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REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. GEN-T121C1
Patent No. 6,822,072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Jean-Baptiste Dumas Milne Edwards, Aymeric Duclert, Jean-Yves Giordano
Issued	:	November 23, 2004
Patent No.	:	6,822,072 09,471,276
For	:	Expressed Sequence Tags and Encoded Human Proteins

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 2, Line 28: "268:7314,"	Page 2, Line 24: --268:731-4,--
Column 4, Line 1: "6:236244,"	Page 4, Line 23: --6:236-244,--
Column 4, Line 3: "Spel"	Page 4, Line 24: --SpeI--

Column 4, Line 4: "Spel"	Page 4, Line 25: --SpeI--
Column 5, Line 19: "tenn"	Page 6, Line 7: --term--
Column 7, Line 61: "extended,cDNAs"	Page 9, Line 16: --extended cDNAs--
Column 8, Line 63: "SEQ D NOs."	Page 10, Line 24: --SEQ ID NOs.--
Column 12, Line 35: "15541580"	Page 15, Line 6: --1554-1580--
Column 20, Line 5: "gene D"	Page 24, Line 32: --gene II--
Column 20, Line 21: "polyerase"	Page 25, Line 8: --polymerase--
Column 21, Line 38: "fill-length"	Page 26, Line 28: --full-length--
Column 25, Line 8: "Ednian"	Page 31, Line 18: --Edman--
Column 25, Line 9: "N-terrrinal"	Page 31, Line 18: --N-terminal--
Column 27, Line 41: "24728"	Page 34, Line 23: --24-728--
Column 30, Line 11: "nucleobde(s)"	Page 37, Line 32: --nucleotide(s)--
Column 31, SEQ ID NO: 415: "53"	Page 40, SEQ ID NO: 415: --S3--
Column 33, SEQ ID NO: 800: "km"	Page 44, SEQ ID NO: 800: --krn--

Column 34, Line 50:
"polynucleolide"

Page 45, Line 15:
--polynucleotide--

Column 45, Line 34:
"5'ESTs Consensus"

Page 63, Lines 3-4:
--5'ESTs, Consensus--

Column 45, Line 53:
"the type"

Page 64, Line 6:
--the tissue type--

Column 54, Lines 12-14:
"647 F:1
643 F:1
649 F:1"

Page 79, Lines 5-7:
--647 F:1
648 F:1
649 F:1--

Column 59, Line 38:
"by.,a"

Page 87, Line 16:
--by a--

Column 60, Line 41:
"it'may"

Page 88, Line 27:
--it may--

Column 61, Line 53:
"bioinfomnatics"

Page 90, Line 11:
--bioinformatics--

Column 67, Line 31:
"403410"

Page 97: Line 19:
--403-410--

Column 68, Line 1:
"17:4941"

Page 98, Line 11:
--17:49-61--

Column 69, Line 61:
"10%-20%=80%"

Page 100, Line 23:
--100%-20%=80%--

Column 70, Line 63:
"Gene H"

Page 101, Line 31:
--Gene II--

Column 72, Line 29:
"10:685686"

Page 103, Line 24:
--10:685-686--

Column 75, Line 6:
"pX1 contains"

Page 107, Line 3:
--pXT1 contains--

Column 75, Line 16:
"BgIII"

Page 107, Line 9:
--BG1II--

Column 77, Line 59:	Page 110, Lines 18-19:
"Cytokine Cell"	--Cytokine, Cell--
Column 78, Line 12:	Page 110, Line 31:
"145:17061712"	--145:1706-1712--
Column 79, Line 36:	Page 112, Line 22:
"Takcai"	--Takai--
Column 79, Line 44:	Page 112, Line 28:
"134:536544"	--134:536-544--
Column 79, Line 47:	Page 112, Line 30:
"67:40624069"	--67:4062-4069--
Column 79, Line 57:	Page 113, Line 3:
"Darzynliewicz"	--Darzynkiewicz--
Column 79, Line 59:	Page 113, Line 4:
"7:659670"	--7:659-670--
Column 81, Line 2:	Page 114, Line 17:
"tsplants"	--transplants--
Column 85, Line 11:	Page 119, Line 21:
"tendonaigament-like"	--tendon/ligament-like--
Column 85, Line 20:	Page 119, Line 27:
"in viva"	--in vivo--
Column 87, Line 55:	Page 123, Line 6:
"45:413419"	--45:413-419--
Column 87, Line 57:	Page 123, Line 7:
"35:467474"	--35:467-474--
Column 89, Line 50:	Page 125, Line 17:
"Inclining"	--killing--
Column 89, Line 63:	Page 125, Line 25:
"behaviora"	--behavioral--

Column 91, Line 48: "*Chromalographia*"	Page 127, Line 30: --*Chromatographia*--
Column 95, Line 40: "GIC"	Page 132, Line 28: --G/C--
Column 95, Line 62: "Acids Positional"	Page 133, Line 8: --Acids, Positional--
Column 97, Line 49: "probes arc"	Page 135, Line 16: --probes are--
Column 98, Line 11: "P2"	Page 135, Line 34: --P^{32}--
Column 98, Line 18: "^{32}p"	Page 136, Line 4: --^{32}P--
Column 98, Line 67: "p^{32}"	Page 137, Line 1: --P^{32}--
Column 99, Line 64: "(FK)"	Page 138, Line 7: --(TK)--
Column 100, Line 9: "Acids Positional"	Page 138, Line 15: --Acids, Positional--
Column 101, Line 40: "Collected"	Page 140, Line 8: --Colcemid--
Column 112, Line 23: "in, both"	Page 153, Line 24: --in both--
Column 113, Line 11: "CIA"	Page 154, Line 26: --CTA--
Column 113, Line 19: "release 20"	Page 154, Line 31: --release 2.0--
Column 115, Line 66: "antiseese"	Page 158, Line 11: --antisense--

Column 119, Line 30: "region (b)"	Page 162, Line 20: --region (h)--
Column 119, Line 61: "271:2745627461"	Page 163, Line 4: --271:27456-27461--
Column 119, Line 63: "234:675680"	Page 163, Line 6: --234:675-680--
Column 120, Line 56: "3^4 edition"	Page 164, Line 27: --3^{rd} edition--
Column 120, Lines 66-67: "990%, 98%, 97%, 96%, 95%, 900%, 85%"	Page 164, Line 34: --99%, 98%, 97%, 96%, 95%, 90%, 85%--
Column 122, Line 18: "Continuing"	Page 166, Line 18: --containing--
Column 124, Lines 60-61: "polynucleotides"	Page 169, Line 26: --polynucleotide--
Column 127, Lines 39-40: "MicrosofRWORD"	Page 173, Line 5: --MicrosoftWORD--
Column 128, Line 49: "desired polynucleotides."	Page 174, Line 17: --desired polynucleotide.--.

A true and correct copy of pages 2, 4, 6, 9, 10, 15, 24-26, 31, 34, 37, 40, 44, 45, 63, 64, 79, 87, 88, 90, 97, 98, 100, 101, 103, 107, 110, 112-114, 119, 123, 125, 127, 132, 133, 135-138, 140, 153, 154, 158, 162-164, 166, 169, 173, and 174 of the specification as filed, which support Applicant's assertion of errors on the part of the Patent Office, accompany this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,

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FCE/sl

Attachments: Certificate of Correction; copies of pages 2, 4, 6, 9, 10, 15, 24-26, 31, 34, 37, 40, 44, 45, 63, 64, 79, 87, 88, 90, 97, 98, 100, 101, 103, 107, 110, 112-114, 119, 123, 125, 127, 132, 133, 135-138, 140, 153, 154, 158, 162-164, 166, 169, 173, and 174 of the specification

addition, the extended cDNAs may contain the full coding sequence of the gene from which the EST was derived or, alternatively, the extended cDNAs may include portions of the coding sequence of the gene from which the EST was derived. It will be appreciated that there may be several extended cDNAs which include the EST sequence as a result of alternate splicing or the activity of alternative promoters. Alternatively, ESTs having partially overlapping sequences may be identified and contigs comprising the consensus sequences of the overlapping ESTs may be identified.

In the past, these short EST sequences were often obtained from oligo-dT primed cDNA libraries. Accordingly, they mainly corresponded to the 3' untranslated region of the mRNA. In part, the prevalence of EST sequences derived from the 3' end of the mRNA is a result of the fact that typical techniques for obtaining cDNAs, are not well suited for isolating cDNA sequences derived from the 5' ends of mRNAs (Adams *et al., Nature* 377:3-174, 1996, Hillier *et al., Genome Res.* 6:807-828, 1996), the entire disclosures of which are incorporated herein by reference.

In addition, in those reported instances where longer cDNA sequences have been obtained, the reported sequences typically correspond to coding sequences and do not include the full 5' untranslated region (5'UTR) of the mRNA from which the cDNA is derived. Indeed, 5'UTRs have been shown to affect either the stability or translation of mRNAs. Thus, regulation of gene expression may be achieved through the use of alternative 5'UTRs as shown, for instance, for the translation of the tissue inhibitor of metalloprotease mRNA in mitogenically activated cells (Waterhouse *et al, J Biol Chem.* **265**:5585-9. 1990) , the entire disclosure of which is incorporated herein by reference. Furthermore, modification of 5'UTR through mutation, insertion or translocation events may even be implied in pathogenesis. For instance, the fragile X syndrome, the most common cause of inherited mental retardation, is partly due to an insertion of multiple CGG trinucleotides in the 5'UTR of the fragile X mRNA resulting in the inhibition of protein synthesis via ribosome stalling (Feng *et al, Science* **268**:731-4, 1995) , the entire disclosure of which is incorporated herein by reference. An aberrant mutation in regions of the 5'UTR known to inhibit translation of the proto-oncogene *c-myc* was shown to result in upregulation of c-myc protein levels in cells derived from patients with multiple myelomas (Willis *et al, Curr Top Microbiol Immunol* **224**:269-76, 1997) , the entire disclosure of which is incorporated herein by reference. In addition, the use of oligo-dT primed cDNA libraries does not allow the isolation of complete 5'UTRs since such incomplete sequences obtained by this process may not include the first exon of the mRNA, particularly in situations where the first exon is short. Furthermore, they may not include some exons, often short ones, which are located upstream of splicing sites. Thus, there is a need to obtain sequences derived from the 5' ends of mRNAs.

undesirable phenotype as a result of a mutation in such a coding sequence, the undesirable phenotype may be corrected by introducing a normal coding sequence using gene therapy. Alternatively, if the undesirable phenotype results from overexpression of the protein encoded by the coding sequence, expression of the protein may be reduced using antisense or triple helix based strategies.

The secreted or non-secreted human polypeptides encoded by the coding sequences may also be used as therapeutics by administering them directly to an individual having a condition, such as a disease, resulting from a mutation in the sequence encoding the polypeptide. In such an instance, the condition can be cured or ameliorated by administering the polypeptide to the individual.

In addition, the secreted or non-secreted human polypeptides or portions thereof may be used to generate antibodies useful in determining the tissue type or species of origin of a biological sample. The antibodies may also be used to determine the cellular localization of the secreted or non-secreted human polypeptides or the cellular localization of polypeptides which have been fused to the human polypeptides. In addition, the antibodies may also be used in immunoaffinity chromatography techniques to isolate, purify, or enrich the human polypeptide or a target polypeptide which has been fused to the human polypeptide.

Public information on the number of human genes for which the promoters and upstream regulatory regions have been identified and characterized is quite limited. In part, this may be due to the difficulty of isolating such regulatory sequences. Upstream regulatory sequences such as transcription factor binding sites are typically too short to be utilized as probes for isolating promoters from human genomic libraries. Recently, some approaches have been developed to isolate human promoters. One of them consists of making a CpG island library (Cross *et al., Nature Genetics* **6**: 236-244, 1994) , the entire disclosure of which is incorporated herein by reference. The second consists of isolating human genomic DNA sequences containing SpeI binding sites by the use of SpeI binding protein. (Mortlock *et al., Genome Res.* **6**:327-335, 1996) , the entire disclosure of which is incorporated herein by reference. Both of these approaches have their limits due to a lack of specificity and of comprehensiveness. Thus, there exists a need to identify and systematically characterize the 5' portions of the genes.

The present 5' ESTs may be used to efficiently identify and isolate 5'UTRs and upstream regulatory regions which control the location, developmental stage, rate, and quantity of protein synthesis, as well as the stability of the mRNA. Once identified and characterized, these regulatory regions may be utilized in gene therapy or protein purification schemes to obtain the desired amount and locations of protein synthesis or to inhibit, reduce, or prevent the synthesis of undesirable gene products.

Preferably, the enriched 5' ESTs represent 15% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules. More preferably, the enriched 5' ESTs represent 50% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules. In a highly preferred embodiment, the enriched 5' ESTs represent 90% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules.

"Stringent," "moderate," and "low" hybridization conditions are as defined below.

The term "polypeptide" refers to a polymer of amino acids without regard to the length of the polymer; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not specify or exclude post-expression modifications of polypeptides, for example, polypeptides which include the covalent attachment of glycosyl groups, acetyl groups, phosphate groups, lipid groups and the like are expressly encompassed by the term polypeptide. Also included within the definition are polypeptides which contain one or more analogs of an amino acid (including, for example, non-naturally occurring amino acids, amino acids which only occur naturally in an unrelated biological system, modified amino acids from mammalian systems etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

As used interchangeably herein, the terms "nucleic acids," "oligonucleotides," and "polynucleotides" include RNA, DNA, or RNA/DNA hybrid sequences of more than one nucleotide in either single chain or duplex form. The term "nucleotide" as used herein as an adjective to describe molecules comprising RNA, DNA, or RNA/DNA hybrid sequences of any length in single-stranded or duplex form. The term "nucleotide" is also used herein as a noun to refer to individual nucleotides or varieties of nucleotides, meaning a molecule, or individual unit in a larger nucleic acid molecule, comprising a purine or pyrimidine, a ribose or deoxyribose sugar moiety, and a phosphate group, or phosphodiester linkage in the case of nucleotides within an oligonucleotide or polynucleotide. Although the term "nucleotide" is also used herein to encompass "modified nucleotides" which comprise at least one modifications (a) an alternative linking group, (b) an analogous form of purine, (c) an analogous form of pyrimidine, or (d) an analogous sugar, for examples of analogous linking groups, purine, pyrimidines, and sugars see for example PCT publication No. WO 95/04064. The polynucleotide sequences of the invention may be prepared by any known method, including synthetic, recombinant, *ex vivo* generation, or a combination thereof, as well as utilizing any purification methods known in the art.

The terms "base paired" and "Watson & Crick base paired" are used interchangeably herein to refer to nucleotides which can be hydrogen bonded to one another be virtue of their sequence identities in a manner like that found in double-helical DNA with thymine or uracil

be referred to hereinafter as "full-length cDNAs." These cDNAs may comprise a 3' untranslated region and eventually a polyadenylation tail. These cDNAs may also include DNA derived from mRNA sequences upstream of the translation start site. The full-length cDNA sequences may be used to express the proteins corresponding to the 5' ESTs. As discussed above, secreted proteins and non-secreted proteins may be therapeutically important. Thus, the proteins expressed from the cDNAs may be useful in treating and controlling a variety of human conditions. The 5' ESTs may also be used to obtain the corresponding genomic DNA. The term "corresponding genomic DNA" refers to the genomic DNA which encodes the mRNA from which the 5' EST was derived.

Alternatively, the 5' ESTs may be used to obtain and express extended cDNAs encoding portions of the protein. In the case of secreted proteins, the portions may comprise the signal peptides of the secreted proteins or the mature proteins generated when the signal peptide is cleaved off.

The present invention includes isolated, purified, or enriched "EST-related nucleic acids." The terms "isolated," "purified" or "enriched" have the meanings provided above. As used herein, the term "EST-related nucleic acids" means the nucleic acids of SEQ ID NOs. 24-811 and 1600-1622, extended cDNAs obtainable using the nucleic acids of SEQ ID NOs. 24-811 and 1600-1622, full-length cDNAs obtainable using the nucleic acids of SEQ ID NOs. 24-811 and 1600-1622 or genomic DNAs obtainable using the nucleic acids of SEQ ID NOs. 24-811 and 1600-1622. The present invention also includes the sequences complementary to the EST-related nucleic acids.

The present invention also includes isolated, purified, or enriched "fragments of EST-related nucleic acids." The terms "isolated," "purified" and "enriched" have the meanings described above. As used herein the term "fragments of EST-related nucleic acids" means fragments comprising at least 10, 12, 15, 18, 20, 23, 25, 28, 30, 35, 40, 50, 75, 100, 200, 300, 500, or 1000 consecutive nucleotides of the EST-related nucleic acids to the extent that fragments of these lengths are consistent with the lengths of the particular EST-related nucleic acids being referenced. In particular, fragments of EST-related nucleic acids refer to "polynucleotides described in Table II," "polynucleotides described in Table III," and "polynucleotides described in Table IV." The present invention also includes the sequences complementary to the fragments of the EST-related nucleic acids.

The present invention also includes isolated, purified, or enriched "positional segments of EST-related nucleic acids." As used herein, the term "positional segments of EST-related nucleic acids" includes segments comprising nucleotides 1-25, 26-50, 51-75, 76-100, 101-125, 126-150, 151-175, 176-200, 201-225, 226-250, 251-300, 301-325, 326-350, 351-375, 376-400, 401-425, 426-450, 451-475, 476-500, 501-525, 526-550, 551-575, 576-600 and 601-the terminal nucleotide of the

EST-related nucleic acids to the extent that such nucleotide positions are consistent with the lengths of the particular EST-related nucleic acids being referenced. The term "positional segments of EST-related nucleic acids also includes segments comprising nucleotides 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 450-500, 501-550, 551-600 or 601-the terminal nucleotide of the EST-related nucleic acids to the extent that such nucleotide positions are consistent with the lengths of the particular EST-related nucleic acids being referenced. The term "positional segments of EST-related nucleic acids" also includes segments comprising nucleotides 1-100, 101-200, 201-300, 301-400, 501-500, 500-600, or 601-the terminal nucleotide of the EST-related nucleic acids to the extent that such nucleotide positions are consistent with the lengths of the particular EST-related nucleic acids being referenced. In addition, the term "positional segments of EST-related nucleic acids" includes segments comprising nucleotides 1-200, 201-400, 400-600, or 601-the terminal nucleotide of the EST-related nucleic acids to the extent that such nucleotide positions are consistent with the lengths of the particular EST-related nucleic acids being referenced. The present invention also includes the sequences complementary to the positional segments of EST-related nucleic acids.

The present invention also includes isolated, purified, or enriched "fragments of positional segments of EST-related nucleic acids." As used herein, the term "fragments of positional segments of EST-related nucleic acids" refers to fragments comprising at least 10, 15, 18, 20, 23, 25, 28, 30, 35, 40, 50, 75, 100, 150, or 200 consecutive nucleotides of the positional segments of EST-related nucleic acids. The present invention also includes the sequences complementary to the fragments of positional segments of EST-related nucleic acids.

The present invention also includes isolated or purified "EST-related polypeptides." As used herein, the term "EST-related polypeptides" means the polypeptides encoded by the EST-related nucleic acids, including the polypeptides of SEQ ID NOs. 812-1599.

The present invention also includes isolated or purified "fragments of EST-related polypeptides." As used herein, the term "fragments of EST-related polypeptides" means fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids of an EST-related polypeptide to the extent that fragments of these lengths are consistent with the lengths of the particular EST-related polypeptides being referenced. In particular, fragments of EST-related polypepides refer to polypeptides encoded by "polynucleotides described in Table II," "polynucleotides described in Table III," and "polynucleotides described in Table IV."

The present invention also includes isolated or purified "positional segments of EST-related polypeptides." As used herein, the term "positional segments of EST-related polypeptides" includes polypeptides comprising amino acid residues 1-25, 26-50, 51-75, 76-100, 101-125, 126-150, 151-

Another embodiment of the present invention is a purified or isolated polypeptide comprising, consisting essentially of, or consisting of a sequence selected from the group consisting of SEQ ID NOs. 1554-1580.

Another embodiment of the present invention is a purified or isolated polypeptide comprising, consisting essentially of, or consisting of a mature protein of a polypeptide selected from the group consisting of SEQ ID NOs. 1554-1580.

Another embodiment of the present invention is a purified or isolated polypeptide comprising, consisting essentially of, or consisting of a signal peptide of a sequence selected from the group consisting of the polypeptides of SEQ ID NOs. 812-1516 and 1554-1580.

Another embodiment of the present invention is a purified or isolated polypeptide comprising, consisting essentially of, or consisting of at least 12, 15, 18, 20, 23, 25, 28, 30, 35, 40, 50, 75, 100, 200, 300, 500, or 1000 consecutive amino acids, to the extent that fragments of these lengths are consistent with the specific sequence, of a sequence selected from the group consisting of the sequences of SEQ ID NOs. 812-1599.

Another embodiment of the present invention is a method of making a cDNA comprising the steps of contacting a collection of mRNA molecules from human cells with a primer comprising at least 12, 15, 18, 20, 23, 25, 28, 30, 35, 40, or 50 consecutive nucleotides of a sequence selected from the group consisting of the sequences complementary to SEQ ID NOs. 24-811 and SEQ ID NOs. 1600-1622, hybridizing said primer to an mRNA in said collection that encodes said protein reverse transcribing said hybridized primer to make a first cDNA strand from said mRNA, making a second cDNA strand complementary to said first cDNA strand and isolating the resulting cDNA encoding said protein comprising said first cDNA strand and said second cDNA strand.

Another embodiment of the present invention is a purified cDNA obtainable by the method of the preceding paragraph.

In one aspect of this embodiment, the cDNA encodes at least a portion of a human polypeptide. Preferably, said human polypeptide comprises at least 8, 10, 12, 15, 18, 20, 23, 25, 28, 30, 35, 40, 50, 75, 100, 200, 300, 500, or 1000 consecutive amino acids, to the extent that fragments of these lengths are consistent with the specific sequence, of a sequence encoded by a sequence selected from the group consisting of the sequences of SEQ ID NOs. 24-811. More preferably, said human polypeptide comprises the polypeptide encoded by a sequence selected from the group consisting of the sequences of SEQ ID NOs. 24-811. In one aspect of this embodiment, said cDNA comprises the complete coding sequence of said human polypeptide.

the oligonucleotide tag to the mRNA, the integrity of the mRNA was then examined by performing a Northern blot using a probe complementary to the oligonucleotide tag.

EXAMPLE 3

cDNA Synthesis Using mRNA Templates Having Intact 5' Ends

For the mRNAs joined to oligonucleotide tags, first strand cDNA synthesis was performed using a reverse transcriptase with random nonamers as primers. In order to protect internal EcoRI sites in the cDNA from digestion at later steps in the procedure, methylated dCTP was used for first strand synthesis. After removal of mRNA by an alkaline hydrolysis, the first strand of cDNA was precipitated using isopropanol in order to eliminate residual primers.

The second strand of the cDNA was synthesized with a Klenow fragment using a primer corresponding to the 5'end of the ligated oligonucleotide. Methylated dCTP was also used for second strand synthesis in order to protect internal EcoRI sites in the cDNA from digestion during the cloning process.

EXAMPLE 4

Cloning of cDNAs derived from mRNA with intact 5' ends into BlueScript

Following second strand synthesis, the ends of the cDNA were blunted with T4 DNA polymerase (Biolabs) and the cDNA was digested with EcoRI. Since methylated dCTP was used during cDNA synthesis, the EcoRI site present in the tag was the only hemi-methylated site, hence the only site susceptible to EcoRI digestion. The cDNA was then size fractionated using exclusion chromatography (AcA, Biosepra) and fractions corresponding to cDNAs of more than 150 bp were pooled and ethanol precipitated. The cDNA was directionally cloned into the SmaI and EcoRI ends of the phagemid pBlueScript vector (Stratagene). The ligation mixture was electroporated into bacteria and propagated under appropriate antibiotic selection.

EXAMPLE 5

Selection of Clones Having the Oligonucleotide Tag Attached Thereto

Clones containing the oligonucleotide tag attached were then selected as follows. The plasmid DNAs containing 5' EST libraries made as described above were purified (Qiagen). A positive selection of the tagged clones was performed as follows. Briefly, in this selection procedure, the plasmid DNA was converted to single stranded DNA using gene II endonuclease of the phage F1 in combination with an exonuclease (Chang *et al.*, **Gene 127**:95-8, 1993) , the entire disclosure of which is incorporated herein by reference, such as exonuclease III or T7 gene 6 exonuclease. The

resulting single stranded DNA was then purified using paramagnetic beads as described by Fry *et al.*, *Biotechniques*, **13**: 124-131, 1992, the entire disclosure of which is incorporated herein by reference. In this procedure, the single stranded DNA was hybridized with a biotinylated oligonucleotide having a sequence corresponding to the 3' end of the oligonucleotide tag. Clones including a sequence complementary to the biotinylated oligonucleotide were captured by incubation with streptavidin coated magnetic beads followed by magnetic selection. After capture of the positive clones, the plasmid DNA was released from the magnetic beads and converted into double stranded DNA using a DNA polymerase such as the Thermosequenase obtained from Amersham Pharmacia Biotech. The double stranded DNA was then electroporated into bacteria. The percentage of positive clones having the 5' tag oligonucleotide was estimated using dot blot analysis to typically be between 90 and 98%.

Following electroporation, the libraries were ordered in 384-microtiter plates (MTP). A copy of the MTP was stored for future needs. Then the libraries were transferred into 96 MTP and sequenced as described below.

EXAMPLE 6

Sequencing of Inserts in Selected Clones

Plasmid inserts were first amplified by PCR on PE-9600 thermocyclers (Perkin-Elmer, Applied Biosystems Division, Foster City, CA), using standard SETA-A and SETA-B primers (Genset SA), AmpliTaqGold (Perkin-Elmer), dNTPs (Boehringer), buffer and cycling conditions as recommended by the Perkin-Elmer Corporation.

PCR products were then sequenced using automatic ABI Prism 377 sequencers (Perkin Elmer). Sequencing reactions were performed using PE 9600 thermocyclers with standard dye-primer chemistry and ThermoSequenase (Amersham Pharmacia Biotech). The primers used were either T7 or 21M13 (available from Genset SA) as appropriate. The primers were labeled with the JOE, FAM, ROX and TAMRA dyes. The dNTPs and ddNTPs used in the sequencing reactions were purchased from Boehringer. Sequencing buffer, reagent concentrations and cycling conditions were as recommended by Amersham.

Following the sequencing reaction, the samples were precipitated with ethanol, resuspended in formamide loading buffer, and loaded on a standard 4% acrylamide gel. Electrophoresis was performed for 2.5 hours at 3000V on an ABI 377 sequencer, and the sequence data were collected and analyzed using the ABI Prism DNA Sequencing Analysis Software, version 2.1.2.

EXAMPLE 7

Obtaining 5' ESTs from Extended cDNA libraries

Obtained from mRNA with Intact 5' Ends

Alternatively, 5'ESTs may be isolated from other cDNA or genomic DNA libraries. Such cDNA or genomic DNA libraries may be obtained from a commercial source or made using other techniques familiar to those skilled in the art. One example of such cDNA library construction, a full-length cDNA library, is as follows.

PolyA+ RNAs are prepared and their quality checked as described in Example 1. Then, the caps at the 5' ends of the polyA+ RNAs are specifically joined to an oligonucleotide tag as described in Example 2. The oligonucleotide tag may contain a restriction site such as Eco RI to facilitate further subcloning procedures. Northern blotting is then performed to check the size of mRNAs having the oligonucleotide tag attached thereto and to ensure that the mRNAs are actually tagged.

First strand synthesis is subsequently carried out for mRNAs joined to the oligonucleotide tag as described in Example 3 above except that the random nonamers are replaced by an oligo-dT primer. For instance, this oligo-dT primer may contain an internal tag of 4 nucleotides which is different from one tissue to the other. Following second strand synthesis using a primer contained in the oligonucleotide tag attached to the 5' end of mRNA, the blunt ends of the obtained double stranded full-length DNAs are modified into cohesive ends to facilitate subcloning. For example, the extremities of full-length cDNAs may be modified to allow subcloning into the Eco RI and Hind III sites of a Bluescript vector using the Eco RI site of the oligonucleotide tag and the addition of a Hind III adaptor to the 3' end of full-length cDNAs.

The full-length cDNAs are then separated into several fractions according to their sizes using techniques familiar to those skilled in the art. For example, electrophoretic separation may be applied in order to yield 3 or 6 different fractions. Following gel extraction and purification, the cDNA fractions are subcloned into appropriate vectors, such as Bluescript vectors, transformed into competent bacteria and propagated under appropriate antibiotic conditions. Subsequently, plasmids containing tagged full-length cDNAs are positively selected as described in Example 5.

The 5' end of full-length cDNAs isolated from such cDNA libraries may then be sequenced as described in Example 6 to yield 5'ESTs.

II. Computer Analysis of the Isolated 5' ESTs: Construction of the SignalTag™ Database

The sequence data from the cDNA libraries made as described above were transferred to a database, where quality control and validation steps were performed. A base-caller, working using a Unix system, automatically flagged suspect peaks, taking into account the shape of the peaks, the

the ORF encoded by the extended cDNA is basically the same as the one encoded by the consensus contigated 5'EST or 5'EST.

Alternatively, to confirm that the chosen ORF actually encodes a polypeptide, the consensus contigated 5'EST or 5'EST may be used to obtain an extended cDNA using any of the techniques described therein, and especially those described in Examples 19 and 20. Such an extended cDNA may then be inserted into an appropriate expression vector and used to express the polypeptide encoded by the extended cDNA as described therein. The expressed polypeptide may be isolated, purified, or enriched as described therein. Several methods known to those skilled in the art may then be used to determine whether the expressed polypeptide is the one actually encoded by the chosen ORF, therein referred to an the expected polypeptide. Such methods are based on the determination of predictable features of the expressed polypeptide, including but not limited to its amino acid sequence, its size or its charge, and the comparison of these features to those predicted for the expected polypeptide. The following paragraphs present examples of such methods.

One of these methods consists in the determination of at least a portion of the amino acid sequence of the expressed polypeptide using any technique known to those skilled in the art. For example, the amino-terminal residues may be determined using techniques either based on Sanger's technique of acid hydrolysis of a polypeptide which N-terminal residue has been covalently labeled or using techniques based on Edman degradation of polypeptides which N-terminal residues are sequentially labeled and cleaved from the polypeptide of interest. The amino acid sequence of the expressed polypeptide may then be compared to the one predicted for the expected polypeptide using any algorithm and parameters described therein.

Alternatively, the size of the expressed polypeptides may be determined using techniques familiar to those skilled in the art such as Coomassie blue or silver staining and subsequently compared to the size predicted for the expected polypeptide. Generally, the band corresponding to the expressed polypeptide will have a mobility near that expected based on the number of amino acids in the open reading frame of the extended cDNA. However, the band may have a mobility different than that expected as a result of modifications such as glycosylation, ubiquitination, or enzymatic cleavage.

Alternatively, specific antibodies or antipeptides may be generated against the expected polypeptide as described in Example 34 and used to perform immunoblotting or immunoprecipitation studies against the expressed polypeptide. The presence of a band in samples from cells containing the expression vector with the extended cDNA which is absent in samples from cells containing the expression vector encoding an irrelevant polypeptide indicates that the expected polypeptide or portion thereof is being expressed. Generally, the band corresponding to the

SEQ ID NOs. 24-728 are nucleic acids having an incomplete ORF which encodes a signal peptide. The locations of the incomplete ORFs and sequences encoding signal peptides are listed in the accompanying Sequence Listing. In addition, the von Heijne score of the signal peptide computed as described in Example 13 is listed as the "score" in the accompanying Sequence Listing. The sequence of the signal-peptide is listed as "seq" in the accompanying Sequence Listing. The "/" in the signal peptide sequence indicates the location where proteolytic cleavage of the signal peptide occurs to generate a mature protein.

SEQ ID NOs. 729-765 are nucleic acids having an incomplete ORF in which no sequence encoding a signal peptide has been identified to date. However, it remains possible that subsequent analysis will identify a sequence encoding a signal peptide in these nucleic acids. The locations of the incomplete ORFs are listed in the accompanying Sequence Listing.

SEQ ID NOs. 766-792 are nucleic acids having a complete ORF which encodes a signal peptide. The locations of the complete ORFs and of the signal peptides, the von Heijne score of the signal peptide, the sequence of the signal-peptide and the proteolytic cleavage site are indicated as described above.

SEQ ID NOs. 793-811 are nucleic acids having a complete ORF in which no sequence encoding a signal peptide has been identified to date. However, it remains possible that subsequent analysis will identify a sequence encoding a signal peptide in these nucleic acids. The locations of the complete ORFs are listed in the accompanying Sequence Listing.

SEQ ID NOs. 812-1516 are "incomplete polypeptide sequences" which include a signal peptide. "Incomplete polypeptide sequences" are polypeptide sequences encoded by nucleic acids in which a start codon has been identified but no stop codon has been identified. These polypeptides are encoded by the nucleic acids of SEQ ID NOs. 24-728. The location of the signal peptide, the von Heijne score of the signal peptide, the sequence of the signal-peptide and the proteolytic cleavage site are indicated as described above.

SEQ ID NOs. 1517-1553 are incomplete polypeptide sequences in which no signal peptide has been identified to date. However, it remains possible that subsequent analysis will identify a signal peptide in these polypeptides. These polypeptides are encoded by the nucleic acids of SEQ ID NOs. 729-765.

SEQ ID NOs. 1554-1580 are "complete polypeptide sequences" which include a signal peptide. "Complete polypeptide sequences" are polypeptide sequences encoded by nucleic acids in which a start codon and a stop codon have been identified. These polypeptides are encoded by the nucleic acids of SEQ ID NOs. 766-792. The location of the signal peptide, the von Heijne score of

fragments are then defined by a range of nucleotide positions from the SEQ IDs of the consensus contigated 5'ESTs as indicated in the second column entitled "positions of preferred fragments." The preferred polynucleotide fragments correspond to the individual 5'ESTs aligned to obtain the consensus contigated 5'EST and to those filed in the priority documents. The third column entitled "variant nucleotides" describes the nucleotide sequence variations observed between the consensus contigated 5'EST and preferred nucleic acid fragments as follows:

A) Substitutions in the sequence of a consensus contigated 5'EST to derive a preferred polynucleotide fragment are denoted by an "S", followed by a number indicating the first nucleotide position in a specific SEQ ID to be substituted in a string of substituted nucleotides or the position of the substituted nucleotide in the case of a single substituted nucleotide. Then there is a coma followed by one or more lower case letters indicating the identity of the nucleotide(s) occurring in the substituted position(s). For example, SEQ ID NO: 3401; Position of preferred fragments: 1-250; Variant nucleotides S45,atc would indicate that a preferred polynucleotide fragment had the sequence of positions 1 to 250 of SEQ ID NO. 3401, except that the nucleotides at positions 45, 46, and 47 were substituted with A, T, and C, respectively, in the preferred polynucleotide as compared with the sequence of SEQ ID No. 3401.

B) Insertions in the sequence of a consensus contigated 5'EST to derive a preferred polynucleotide fragment are denoted by an "I", followed by a number indicating the nucleotide position in a specific SEQ ID after which a string of nucleotides is inserted or the position after which the nucleotide is inserted in the case of a single inserted nucleotide. Then there is a coma followed by one or more lower case letters indicating the identity of the nucleotide(s) occurring in the inserted position(s). For example, SEQ ID NO: 7934; Position of preferred fragments: 1-500; Variant nucleotides: I36,gataca would indicate that a preferred polynucleotide fragment had the sequence of positions 1 to 500 of SEQ ID NO. 7934, except that after the nucleotides at position 36 a GATACA string of nucleotides is inserted in the preferred polynucleotide as compared with the sequence of SEQ ID No. 7934.

C) Deletions in the sequence of a consensus contigated 5'EST to derive a preferred nucleic acid fragment are denoted by an "D", followed by a number indicating the first nucleotide position in a specific SEQ ID to be deleted in a string of deleted nucleotides or the position of the deleted nucleotide in the case of a single deleted nucleotide. Then there is a coma followed by number indicating the number of nucleotide(s) deleted from the sequence provided in the sequence ID. For example, SEQ ID NO: 5398; Position of preferred fragments: 56-780; Variant nucleotides D114,5 would indicate that a preferred

SEQ ID NO.	Positions of Preferred Fragments	Variant nucleotides
194	1-215	S50, s; S186, sn; S199, k; I215, gcagcggg
213	1-158	S128, m; I132, w; S143, d; I158, tgcccggg
223	3-431	D1, 2; S28, s; S79, c; S82, s; S308, nr; S328, nb; I431, ccggc
247	1-359	I76, gttt; I359, tccctgg
258	1-236	S72, r; S81, g; S197, s; I205, ss; S232, k; I236, acttcggg
264	5-283	D1, 4; S64, g; S122, m; S134, yy; I137, c; I151, t; I283, gttgc
269	1-143	S111, s; I143, ggggcggg
286	5-207	D1, 4; S204, a; S206, c; I207, gg; D208, 567
287	1-277	S114, r; I125, t; S131, ag; S256, tg; S259, tt; S262, at; S267, t; S269, c; S273, c; I277, ccggg; D278, 337
289	69-416	D1, 68; I416, agccaggg
289	1-278	S114, r; I125, t; S131, ag; S277, c; I278, cggg; D279, 138
292	20-254	D1, 19; I254, aaagagg
293	1-414	I414, tagcag
300	1-285	S16, m; S67, y; I285, baccacggg; D286, 1
349	23-431	D1, 22; I118, a; S214, y; I431, caactgg
350	3-386	D1, 2; S42, w; I263, c; I386, gggat
368	3-446	D1, 2; I446, tctct
385	1-193	I35, t; I108, t; I134, r; S135, a; S137, r; S143, w; I178, c; I193, gagcgggg
411	6-391	D1, 5; S17, r; S27, t; S334, y; D392, 244
412	1-185	S49, s; S127, s; I185, gctggg; D186, 150
415	2-229	D1, 1; S3, a; I229, caaatggg
435	1-386	S4, s; I386, ccggg
436	4-472	D1, 3; S61, sa; D238, 1; S239, s; I472, agtgtgg
437	1-340	I340, ggg; D341, 129
441	1-409	S109, smag; I409, cgcacggg
454	1-492	S72, nn; S115, t; S121, bwy; S181, yn; I492, gagtc
455	1-177	I14, w; I16, a; I177, gagctggg
459	1-311	S39, n; S74, rg; I311, accatggg
460	1-425	I425, agtac
461	5-420	D1, 4; I420, tcgtc
481	1-429	I10, w; S262, d; S333, n; I429, ctccaggg
489	1-414	D72, 1; S117, n; S396, d; I414, ggaca
496	1-215	I215, ttttcggg
501	1-430	S275, n; I430, aggat
502	91-413	D1, 90; I413, aaacgggg

SEQ ID NO.	Positions of Preferred Fragments	Variant nucleotides
797	1-420	S136, c; S150, c; I245, ccc; I420, ggagtg
798	25-316	D1, 24; S315, g; D317, 97
799	1-344	D345, 57
800	7-465	D1, 6; S59, k; S146, a; S186, krn; I465, gttca
801	121-422	D1, 120; I269, c; S419, cc; I422, gg; D423, 207
802	46-477	D1, 45; S132, bn; I477, actac
803	15-467	D1, 14; S45, k; S65, t; S418, ys; D452, 1; D468, 119
804	1-341	S42, t; S97, d; S326, gtg; S331, tgt; S336, a; S338, c; I341, ccccgggg; D342, 218
805	2-409	D1, 1; S334, d; I409, aggg; D410, 161
806	5-384	D1, 4; I384, actaa
807	1-301	S113, a; S117, c; S123, t; D128, 1; D134, 1; S282, g; S284, a; I301, gacggagggg; D302, 70
808	2-314	D1, 1; S306, g; I314, ggg; D315, 121
809	1-394	S53, g; S228, n; S272, vk; I301, g; I358, m; S368, nb; S375, w; I383, mm; I388, yt; I394, nhaccggg
810	6-205	I0, a; D1, 5; I141, t; I205, ggg; D206, 630
811	6-270	D1, 5; I270, gggg; D271, 115
1600	1-247	S45, m; S114, k; I122, m; S123, yc; S158, rr; S221, k; I247, ccccaggg
1601	1-225	S109, bm; S195, m; I225, tgcacggg
1602	23-245	D1, 22; D138, 1; S139, s; S242, t; S244, g; I245, g; D246, 13
1603	1-303	S71, c; D277, 1; I303, ggagggg; D304, 38
1604	1-242	S47, w; S50, c; S81, h; S85, d; S91, k; S106, r; I242, tgtggg; D243, 50
1605	2-225	D1, 1; S20, k; S91, c; I225, ggg; D226, 132
1606	15-293	D1, 14; S156, g; S193, g; I200, t; I293, acaaaggg
1607	1-361	S323, c; I361, cccca
1608	1-151	I151, taagggg; D152, 154
1609	1-242	S55, s; I135, a; S152, h; I242, cagtaggg
1610	1-196	I151, w; S190, k; I196, cctgtgg
1611	1-228	S115, k; S174, rk; I228, cgtttggg
1612	1-221	S108, v; I221, tgatcggg
1613	1-281	I66, w; I137, a; D282, 79
1614	1-171	S53, k; S76, k; I80, k; S81, kw; S86, r; S92, k; S126, k; I171, gccgagg
1615	2-193	D1, 1; S67, c; I121, s; S122, mm; S126, g; S130, r; S146, r; S156, gm; I193, cctca

SEQ ID NO.	Positions of Preferred Fragments	Variant nucleotides
1616	1-349	S251, ww; S259, rs; S275, k; I279, w; S285, y; S292, y; I320, m; I331, m; I338, w; I341, s; I349, accccggg
1617	1-129	I118, t; D130, 26
1618	1-184	D9, 1; D185, 1
1619	1-169	I122, t; I169, gcccaggg
1620	1-187	S106, k; S118, m; S122, cg; S132, k; D188, 59
1621	1-153	D125, 1; I131, ttt; S152, t; I153, gg; D154, 127
1622	1-400	S43, s; I126, g; I129, y; S353, d; I400, tatat

EXAMPLE 16

Categorization of 5' ESTs and Consensus Contigated 5'ESTs

The nucleic acid sequences of the present invention (SEQ ID NOs. 24-811 and 1600-1622) were grouped based on their homology to known sequences as follows. All sequences were compared to EMBL release 57 and daily releases available at the time of filing using BLASTN. All matches with a minimum of 25 nucleotides with 90% homology were retrieved and used to compute Tables III and IV.

In some embodiments, 5'ESTs or consensus contigated 5'ESTs nucleic acid sequence do not match any known vertebrate sequence nor any publicly available EST sequence, thus being completely new.

In other embodiments, 5'ESTs or consensus contigated 5'ESTs match a known sequence. Tables III and IV gives for each sequence of the invention in this category referred to by its sequence identification number in the first column, the positions of their preferred fragments in the second column entitled "Positions of preferred fragments." As used herein the term "polynucleotide described in Table III" refers to the all of the preferred polynucleotide fragments defined in Table III in this manner, and the term "polynucleotide described in Table IV" refers to the all of the preferred polynucleotides fragments defined in Table IV in this manner. The present invention encompasses isolated, purified, or recombinant nucleic acids which consist of, consist essentially of, or comprise a contiguous span of at least 8, 10, 12, 15, 18, 20, 25, 35, 40, 50, 70, 80, 100, 250, or 500 nucleotides in length, to the extent that a contiguous span of these lengths is consistent with the lengths of the particular polynucleotide, of a polynucleotide described in Table III or Table IV, or a sequence complementary thereto, wherein said polynucleotide described in Table III or Table IV is selected individually or in any combination from the polynucleotides described in Table III or Table IV. The present invention also encompasses isolated, purified, or recombinant nucleic acids which

SEQ ID NO	Positions of Preferred Fragments
782	1-59
783	1-53
784	1-220, 262-390
785	1-339, 408-461
786	1-28
789	1-58
791	1-126
792	1-31, 129-220
793	1-31
794	355-431
795	1-33
797	1-31
798	1-31
799	1-401
801	1-117
802	1-92
806	64-384
807	1-331
808	1-351
810	1-39
1600	1-25
1603	1-341
1606	1-31
1607	1-361
1608	164-305
1611	85-228
1612	1-221
1613	112-360
1614	1-171
1615	94-193
1617	1-155
1620	1-246

III. Evaluation of Spatial and Temporal Expression of mRNAs Corresponding to the 5'ESTs, Consensus Contigated 5'ESTs, or EST-related nucleic acids

5

EXAMPLE 17

Expression Patterns of mRNAs From Which the 5'ESTs were obtained

Each of the SEQ ID NOs. 24-811 and 1600-1622 was also categorized based on the tissue from which its corresponding mRNA was obtained, as follows.

Table V shows the spatial distribution of each nucleic acid sequence of the invention (SEQ ID NOs. 24-811 and 1600-1622) referred to by its sequence identification number in the first column. In the second column entitled tissue distribution, the spatial distribution is represented by the number of individual 5'ESTs used to assemble the consensus contigated 5'ESTs for a given tissue. Each type of tissue listed in Table V is encoded by a letter. The correspondence between the letter code and the tissue type is given in Table VI.

Table V

SEQ ID NO	Tissue Distribution
24	AA:1
25	S:1
26	P:1
27	W:1
28	P:1
29	S:1
30	P:1
31	P:1
32	P:1
33	P:1
34	AB:1
35	G:3; P:1; S:1; W:3; AA:4
36	P:1
37	S:1
38	Q:1
39	P:1
40	AB:1
41	B:1; C:3; F:1; G:1; H:4; S:2; T:8; W:1; Z:1; AA:3; AC:1; AD:3
42	A:1
43	N:2
44	P:1
45	C:2; K:1; O:1; S:5
46	K:1; S:2; AA:1
47	AA:1
48	C:1; O:1; P:8
49	P:1
50	P:1
51	P:1
52	S:1
53	AA:1
54	T:1
55	P:1
56	P:1

SEQ ID NO	Tissue Distribution
644	AA:1
645	T:1
646	K:1
647	F:1
648	F:1
649	F:1
650	T:1
651	W:1
652	T:1
653	T:1
654	P:1
655	B:1; H:2; N:1; T:3; Y:1
656	B:1
657	T:1
658	R:1
659	K:1
660	W:1
661	AA:1
662	Y:1
663	W:1
664	G:1
665	S:1
666	Y:1
667	F:1
668	T:1
669	B:1
670	F:1
671	T:1
672	A:2; B:6; C:1; G:1; H:3; J:1; L:1; P:2; Q:1; S:4; T:1; V:3; W:2; Y:1; AA:3; AD:2
673	T:1
674	G:1
675	F:1
676	M:1
677	G:1
678	Y:1
679	D:1
680	P:1
681	D:1
682	AA:1
683	G:1
684	K:1

EST-related nucleic acids, fragments of EST-related nucleic acids, positional segments EST-related nucleic acids, or fragments of positional segments of EST-related nucleic acids may be more than 500 nucleotides long.

For example, quantitative analysis of gene expression may be performed with EST-related nucleic acids, fragments of EST-related nucleic acids, positional segments EST-related nucleic acids, or fragments of positional segments of EST-related nucleic acids in a complementary DNA microarray as described by Schena *et al.* (*Science* 270:467-470, 1995; *Proc. Natl. Acad. Sci. U.S.A.* 93:10614-10619, the entire disclosure of which is incorporated herein by reference, 1996). EST-related nucleic acids, fragments of EST-related nucleic acids, positional segments EST-related nucleic acids, or fragments of positional segments of EST-related nucleic acids are amplified by PCR and arrayed from 96-well microtiter plates onto silylated microscope slides using high-speed robotics. Printed arrays are incubated in a humid chamber to allow rehydration of the array elements and rinsed, once in 0.2% SDS for 1 min, twice in water for 1 min and once for 5 min in sodium borohydride solution. The arrays are submerged in water for 2 min at 95°C, transferred into 0.2% SDS for 1 min, rinsed twice with water, air dried and stored in the dark at 25°C.

Cell or tissue mRNA is isolated or commercially obtained and probes are prepared by a single round of reverse transcription. Probes are hybridized to 1 cm^2 microarrays under a 14 x 14 mm glass coverslip for 6-12 hours at 60°C. Arrays are washed for 5 min at 25°C in low stringency wash buffer (1 x SSC/0.2% SDS), then for 10 min at room temperature in high stringency wash buffer (0.1 x SSC/0.2% SDS). Arrays are scanned in 0.1 x SSC using a fluorescence laser scanning device fitted with a custom filter set. Accurate differential expression measurements are obtained by taking the average of the ratios of two independent hybridizations.

Quantitative analysis of the expression of genes may also be performed with EST-related nucleic acids, fragments of EST-related nucleic acids, positional segments EST-related nucleic acids, or fragments of positional segments of EST-related nucleic acids in complementary DNA arrays as described by Pietu *et al.* (*Genome Research* 6:492-503, 1996) , the entire disclosure of which is incorporated herein by reference. The EST-related nucleic acids, fragments of EST-related nucleic acids, positional segments EST-related nucleic acids, or fragments of positional segments of EST-related nucleic acids thereof are PCR amplified and spotted on membranes. Then, mRNAs originating from various tissues or cells are labeled with radioactive nucleotides. After hybridization and washing in controlled conditions, the hybridized mRNAs are detected by phospho-imaging or autoradiography. Duplicate experiments are performed and a quantitative analysis of differentially expressed mRNAs is then performed.

Alternatively, expression analysis of the EST-related nucleic acids, fragments of EST-related nucleic acids, positional segments EST-related nucleic acids, or fragments of positional segments of EST-related nucleic acids can be done through high density nucleotide arrays as described by Lockhart *et al.* (*Nature Biotechnology* **14**: 1675-1680, 1996) and Sosnowsky *et al.* (*Proc. Natl. Acad. Sci.* **94**:1119-1123, 1997) , the entire disclosures of which are incorporated herein by reference. Oligonucleotides of 15-50 nucleotides corresponding to sequences of EST-related nucleic acids, fragments of EST-related nucleic acids, positional segments EST-related nucleic acids, or fragments of positional segments of EST-related nucleic acids are synthesized directly on the chip (Lockhart *et al.*, *supra*) or synthesized and then addressed to the chip (Sosnowsky *et al.*, *supra*). Preferably, the oligonucleotides are about 20 to 25 nucleotides in length.

cDNA probes labeled with an appropriate compound, such as biotin, digoxigenin or fluorescent dye, are synthesized from the appropriate mRNA population and then randomly fragmented to an average size of 50 to 100 nucleotides. The said probes are then hybridized to the chip. After washing as described in Lockhart *et al*, *supra* and application of different electric fields (Sonowsky et *al*, *supra*.), the dyes or labeling compounds are detected and quantified. Duplicate hybridizations are performed. Comparative analysis of the intensity of the signal originating from cDNA probes on the same target oligonucleotide in different cDNA samples indicates a differential expression of the mRNA corresponding to the 5' EST, consensus contigated 5' EST or extended cDNA from which the oligonucleotide sequence has been designed.

IV. Use of 5' ESTs to Clone Extended cDNAs and to Clone the Corresponding Genomic DNAs

Once 5' ESTs or consensus contigated 5' ESTs which include the 5' end of the corresponding mRNAs have been selected using the procedures described above, they can be utilized to isolate extended cDNAs which contain sequences adjacent to the 5' ESTs or consensus contigated 5' ESTs. The extended cDNAs may include the entire coding sequence of the protein encoded by the corresponding mRNA, including the authentic translation start site. If the extended cDNA encodes a secreted protein, it may contain the signal sequence, and the sequence encoding the mature protein remaining after cleavage of the signal peptide.

Extended cDNAs which include the entire coding sequence of the protein encoded by the corresponding mRNA are referred to herein as "full-length cDNAs." Alternatively, the extended cDNAs may not include the entire coding sequence of the protein encoded by the corresponding mRNA, although they do include sequences adjacent to the 5'ESTs or consensus contigated 5' ESTs. In some embodiments in which the extended cDNAs are derived from an mRNA encoding a secreted

After removal of the mRNA hybridized to the first cDNA strand by alkaline hydrolysis, the products of the alkaline hydrolysis and the residual poly dT primer can be eliminated with an exclusion column.

Subsequently, a pair of nested primers on each end is designed based on the known 5' sequence from the 5' EST or consensus contigated 5' EST and the known 3' end added by the poly dT primer used in the first strand synthesis. Software used to design primers are either based on GC content and melting temperatures of oligonucleotides, such as OSP (Illier and Green, *PCR Meth. Appl.* 1:124-128, 1991), the entire disclosure of which is incorporated herein by reference, or based on the octamer frequency disparity method (Griffais *et al.*, *Nucleic Acids Res.* 19: 3887-3891, 1991), the entire disclosure of which is incorporated herein by reference such as PC-Rare (http:// bioinformatics.weizmann.ac.il/software/PC-Rare/doc/manuel.html). Preferably, the nested primers at the 5' end and the nested primers at the 3' end are separated from one another by four to nine bases. These primer sequences may be selected to have melting temperatures and specificities suitable for use in PCR.

A first PCR run is performed using the outer primer from each of the nested pairs. A second PCR run using the inner primer from each of the nested pairs is then performed on a small sample of the first PCR product. Thereafter, the primers and remaining nucleotide monomers are removed.

2. Sequencing Extended cDNAs or Fragments Thereof

Due to the lack of position constraints on the design of 5' nested primers compatible for PCR use using the OSP software, amplicons of two types are obtained. Preferably, the second 5' primer is located upstream of the translation initiation codon thus yielding a nested PCR product containing the entire coding sequence. Such an extended cDNA may be used in a direct cloning procedure as described in section a below. However, in some cases, the second 5' primer is located downstream of the translation initiation codon, thereby yielding a PCR product containing only part of the ORF. Such incomplete PCR products are submitted to a modified procedure described in section b below.

a) Nested PCR products containing complete ORFs

When the resulting nested PCR product contains the complete coding sequence, as predicted from the 5'EST or consensus contigated 5' EST sequence, it is directly cloned in an appropriate vector as described in section 3.

b) Nested PCR products containing incomplete ORFs

When the amplicon does not contain the complete coding sequence, intermediate steps are necessary to obtain both the complete coding sequence and a PCR product containing the full

computer readable medium as described below and compared to one another using any of a variety of algorithms familiar to those skilled in the art, those described below.

To determine the level of homology between the polypeptide encoded by the hybridizing cDNA or genomic DNA and the polypeptide encoded by the 5'EST, consensus contigated 5'EST or extended cDNA from which the probe was derived, the polypeptide sequence encoded by the hybridized nucleic acid and the polypeptide sequence encoded by the 5'EST, consensus contigated 5'EST or extended cDNA from which the probe was derived are compared. The sequences of the polypeptide encoded by the 5'EST, consensus contigated 5'EST or extended cDNA from which the probe was derived and the polypeptide sequence encoded by the cDNA or genomic DNA which hybridized to the detectable probe may be stored on a computer readable medium as described below and compared to one another using any of a variety of algorithms familiar to those skilled in the art, those described below.

Protein and/or nucleic acid sequence homologies may be evaluated using any of the variety of sequence comparison algorithms and programs known in the art. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW (Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci. USA 85(8)*:2444-2448; Altschul *et al.*, 1990, *J. Mol. Biol. 215(3)*:403-410; Thompson *et al.*, 1994, *Nucleic Acids Res. 22(2)*:4673-4680; Higgins *et al.*, 1996, *Methods Enzymol. 266*:383-402; Altschul *et al.*, 1990, *J. Mol. Biol. 215(3)*:403-410; Altschul *et al.*, 1993, *Nature Genetics 3*:266-272) , the entire disclosures of which are incorporated herein by reference.

In a particularly preferred embodiment, protein and nucleic acid sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST") which is well known in the art (see, *e.g.*, Karlin and Altschul, 1990, *Proc. Natl. Acad. Sci. USA 87*:2267-2268; Altschul *et al.*, 1990, *J. Mol. Biol. 215*:403-410; Altschul *et al.*, 1993, *Nature Genetics 3*:266-272; Altschul *et al.*, 1997, *Nuc. Acids Res. 25*:3389-3402) , the entire disclosures of which are incorporated herein by reference. In particular, five specific BLAST programs are used to perform the following task:

(1) BLASTP and BLAST3 compare an amino acid query sequence against a protein sequence database;

(2) BLASTN compares a nucleotide query sequence against a nucleotide sequence database;

(3) BLASTX compares the six-frame conceptual translation products of a query nucleotide sequence (both strands) against a protein sequence database;

(4) TBLASTN compares a query protein sequence against a nucleotide sequence database translated in all six reading frames (both strands); and

(5) TBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

The BLAST programs identify homologous sequences by identifying similar segments, which are referred to herein as "high-scoring segment pairs," between a query amino or nucleic acid sequence and a test sequence which is preferably obtained from a protein or nucleic acid sequence database. High-scoring segment pairs are preferably identified (*i.e.*, aligned) by means of a scoring matrix, many of which are known in the art. Preferably, the scoring matrix used is the BLOSUM62 matrix (Gonnet *et al.*, 1992, *Science 256*:1443-1445; Henikoff and Henikoff, 1993, *Proteins 17*:49-61) , the entire disclosures of which are incorporated herein by reference. Less preferably, the PAM or PAM250 matrices may also be used (see, *e.g.*, Schwartz and Dayhoff, eds., 1978, *Matrices for Detecting Distance Relationships: Atlas of Protein Sequence and Structure*, Washington: National Biomedical Research Foundation), the entire disclosure of which is incorporated herein by reference.

The BLAST programs evaluate the statistical significance of all high-scoring segment pairs identified, and preferably selects those segments which satisfy a user-specified threshold of significance, such as a user-specified percent homology. Preferably, the statistical significance of a high-scoring segment pair is evaluated using the statistical significance formula of Karlin (see, *e.g.*, Karlin and Altschul, 1990, *Proc. Natl. Acad. Sci. USA 87*:2267-2268) , the entire disclosure of which is incorporated herein by reference.

The parameters used with the above algorithms may be adapted depending on the sequence length and degree of homology studied. In some embodiments, the parameters may be the default parameters used by the algorithms in the absence of instructions from the user.

In some embodiments, the level of homology between the hybridized nucleic acid and the extended cDNA, 5'EST, or 5' consensus contigated 5'EST from which the probe was derived may be determined using the FASTDB algorithm described in Brutlag *et al.* Comp. App. Biosci. 6:237-245, 1990, the entire disclosure of which is incorporated herein by reference. In such analyses the parameters may be selected as follows: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the sequence which hybridizes to the probe, whichever is shorter. Because the FASTDB program does not consider 5' or 3' truncations when calculating homology levels, if the sequence which hybridizes to the probe is truncated relative to the sequence of the extended cDNA, 5'EST, or consensus contigated 5'EST from which the probe was

Alternatively, the level of polypeptide homology may be determined using the FASTDB algorithm described by Brutlag *et al.* Comp. App. Biosci. 6:237-245, 1990, the entire disclosure of which is incorporated herein by reference. In such analyses the parameters may be selected as follows: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=Sequence Length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the homologous sequence, whichever is shorter. If the homologous amino acid sequence is shorter than the amino acid sequence encoded by the extended cDNA, 5'EST, or consensus contigated 5' EST as a result of an N terminal and/or C terminal deletion the results may be manually corrected as follows. First, the number of amino acid residues of the amino acid sequence encoded by the extended cDNA, 5'EST, or consensus contigated 5' EST which are not matched or aligned with the homologous sequence is determined. Then, the percentage of the length of the sequence encoded by the extended cDNA, 5'EST, or consensus contigated 5' EST which the non-matched or non-aligned amino acids represent is calculated. This percentage is subtracted from the homology level. For example wherein the amino acid sequence encoded by the extended cDNA, 5'EST, or consensus contigated 5' EST is 100 amino acids in length and the length of the homologous sequence is 80 amino acids and wherein the amino acid sequence encoded by the extended cDNA or 5'EST is truncated at the N terminal end with respect to the homologous sequence. the homology level is calculated as follows. In the preceding scenario there are 20 non-matched, non-aligned amino acids in the sequence encoded by the extended cDNA, 5'EST, or consensus contigated 5' EST. This represents 20% of the length of the amino acid sequence encoded by the extended cDNA, 5'EST, or consensus contigated 5' EST. If the remaining amino acids are 1005 identical between the two sequences, the homology level would be 100%-20%=80% homology. No adjustments are made if the non-matched or non-aligned sequences are internal or under any other conditions.

In addition to the above described methods, other protocols are available to obtain extended cDNAs using 5' ESTs or consensus contigated 5'ESTs as outlined in the following paragraphs.

Extended cDNAs may be prepared by obtaining mRNA from the tissue, cell, or organism of interest using mRNA preparation procedures utilizing polyA selection procedures or other techniques known to those skilled in the art. A first primer capable of hybridizing to the polyA tail of the mRNA is hybridized to the mRNA and a reverse transcription reaction is performed to generate a first cDNA strand.

The first cDNA strand is hybridized to a second primer containing at least 10 consecutive nucleotides of the sequences of SEQ ID NOs 24-811 and 1600-1622. Preferably, the primer comprises at least 10, 12, 15, 17, 18, 20, 23, 25, or 28 consecutive nucleotides from the sequences of

SEQ ID NOs 24-811 and 1600-1622. In some embodiments, the primer comprises more than 30 nucleotides from the sequences of SEQ ID NOs 24-811 and 1600-1622. If it is desired to obtain extended cDNAs containing the full protein coding sequence, including the authentic translation initiation site, the second primer used contains sequences located upstream of the translation initiation site. The second primer is extended to generate a second cDNA strand complementary to the first cDNA strand. Alternatively, RT-PCR may be performed as described above using primers from both ends of the cDNA to be obtained.

Extended cDNAs containing 5' fragments of the mRNA may be prepared by hybridizing an mRNA comprising the sequences of SEQ ID NOs. 24-811 and 1600-1622 with a primer comprising a complementary to a fragment of an EST-related nucleic acid hybridizing the primer to the mRNAs, and reverse transcribing the hybridized primer to make a first cDNA strand from the mRNAs. Preferably, the primer comprises at least 10, 12, 15, 17, 18, 20, 23, 25, or 28 consecutive nucleotides of the sequences complementary to SEQ ID NOs. 24-811 and 1600-1622.

Thereafter, a second cDNA strand complementary to the first cDNA strand is synthesized. The second cDNA strand may be made by hybridizing a primer complementary to sequences in the first cDNA strand to the first cDNA strand and extending the primer to generate the second cDNA strand.

The double stranded extended cDNAs made using the methods described above are isolated and cloned. The extended cDNAs may be cloned into vectors such as plasmids or viral vectors capable of replicating in an appropriate host cell. For example, the host cell may be a bacterial, mammalian, avian, or insect cell.

Techniques for isolating mRNA, reverse transcribing a primer hybridized to mRNA to generate a first cDNA strand, extending a primer to make a second cDNA strand complementary to the first cDNA strand, isolating the double stranded cDNA and cloning the double stranded cDNA are well known to those skilled in the art and are described *in Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. 1997 and Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989.

Alternatively, other procedures may be used for obtaining full-length cDNAs or extended cDNAs. In one approach, full-length or extended cDNAs are prepared from mRNA and cloned into double stranded phagemids as follows. The cDNA library in the double stranded phagemids is then rendered single stranded by treatment with an endonuclease, such as the Gene II product of the phage F1 and an exonuclease (Chang *et al.*, *Gene* 127:95-8, 1993) , the entire disclosure of which is incorporated herein by reference. A biotinylated oligonucleotide comprising the sequence of a fragment of an EST-related nucleic acid is hybridized to the single stranded phagemids. Preferably,

that were screened for the presence of known protein signatures and motifs using the Proscan software from the GCG package and the Prosite 15.0 database are provided below.

The protein of SEQ ID NO: 8 encoded by the full-length cDNA SEQ ID NO: 7 (internal designation 78-8-3-E6-CL0_1C) and expressed in adult prostate belong to the phosphatidylethanolamine-binding protein from which it exhibits the characteristic PROSITE signature from positions 90 to 112. Proteins from this widespread family, from nematodes to fly, yeast, rodent and primate species, bind hydrophobic ligands such as phospholipids and nucleotides. They are mostly expressed in brain and in testis and are thought to play a role in cell growth and/or maturation, in regulation of the sperm maturation, motility and in membrane remodeling. They may act either through signal transduction or through oxidoreduction reactions (for a review see Schoentgen and Jollès, *FEBS Letters*, 369:22-26 (1995), the entire disclosure of which is incorporated herein by reference). Taken together, these data suggest that the protein of SEQ ID NO: 8 may play a role in cell growth, maturation and in membrane remodeling and/or may be related to male fertility. Thus, these protein may be useful in diagnosing and/or treating cancer, neurodegenerative diseases, and/or disorders related to male fertility and sterility.

The protein of SEQ ID No. 10 encoded by the full-length cDNA SEQ ID N0. 9 (internal designation 108-013-5-O-H9-FLC) shows homologies with a family of lysophospholipases conserved among eukaryotes (yeast, rabbit, rodents and human). In addition, some members of this family exhibit a calcium-independent phospholipase A2 activity (Portilla *et al. J. Am. Soc. Nephro.*, 9 :1178-1186 (1998), the entire disclosure of which is incorporated herein by reference). All members of this family exhibit the active site consensus GXSXG motif of carboxylesterases that is also found in the protein of SEQ ID N0. 10 (position 54 to 58). In addition, this protein may be a membrane protein with one transmembrane domain as predicted by the software TopPred II (Claros and von Heijne, *CABIOS applic. Notes*, 10 :685-686 (1994), the entire disclosure of which is incorporated herein by reference). Taken together, these data suggest that the protein of SEQ ID NO:10 may play a role in fatty acid metabolism, probably as a phospholipase. Thus, this protein or part therein, may be useful in diagnosing and/or treating several disorders including, but not limited to, cancer, diabetes, and neurodegenerative disorders such as Parkinson's and Alzheimer's diseases. It may also be useful in modulating inflammatory responses to infectious agents and/or to suppress graft rejection.

The protein of SEQ ID NO: 12 encoded by the full-length cDNA SEQ ID NO: 11 (internal designation 108-004-5-0-D10-FLC) shows remote homology to a subfamily of beta4-galactosyltransferases widely conserved in animals (human, rodents, cow and chicken). Such enzymes, usually type II membrane proteins located in the endoplasmic reticulum or in the Golgi

polyA signal, this sequence can be added to the construct by, for example, splicing out the polyA signal from pSG5 (Stratagene) using BglI and SalI restriction endonuclease enzymes and incorporating it into the mammalian expression vector pXT1 (Stratagene). pXT1 contains the LTRs and a portion of the *gag* gene from Moloney Murine Leukemia Virus. The position of the LTRs in the construct allow efficient stable transfection. The vector includes the Herpes Simplex thymidine kinase promoter and the selectable neomycin gene. The nucleic acid encoding the polypeptide to be expressed is obtained by PCR from the bacterial vector using oligonucleotide primers complementary to the nucleic acid encoding the protein or polypeptide to be expressed and containing restriction endonuclease sequences for Pst I incorporated into the 5'primer and BglII at the 5' end of 3' primer, taking care to ensure that the nucleic acid encoding the protein or polypeptide to be expressed is correctly positioned with respect to the poly A signal. The purified fragment obtained from the resulting PCR reaction is digested with PstI, blunt ended with an exonuclease, digested with Bgl II, purified and ligated to pXT1, now containing a poly A signal and digested with BglII.

The ligated product is transfected into mouse NIH 3T3 cells using Lipofectin (Life Technologies, Inc., Grand Island, New York) under conditions outlined in the product specification. Positive transfectants are selected after growing the transfected cells in 600 μg/ml G418 (Sigma, St. Louis, Missouri).

Alternatively, the nucleic acid encoding the protein or polypeptide to be expressed may be cloned into pED6dpc2. The resulting pED6dpc2 constructs may be transfected into a suitable host cell, such as COS 1 cells. Methotrexate resistant cells are selected and expanded. The expressed protein or polypeptide may be isolated, purified, or enriched as described above.

To confirm expression of the desired protein or polypeptide, the proteins or polypeptides produced by cells containing a vector with a nucleic acid insert encoding the protein or polypeptide are compared to those lacking such an insert. The expressed proteins are detected using techniques familiar to those skilled in the art such as Coomassie blue or silver staining or using antibodies against the protein or polypeptide encoded by the nucleic acid insert. Antibodies capable of specifically recognizing the protein of interest may be generated using synthetic 15-mer peptides having a sequence encoded by the appropriate nucleic acid. The synthetic peptides are injected into mice to generate antibody to the polypeptide encoded by the nucleic acid.

If the proteins or polypeptides encoded by the nucleic acid inserts are secreted, medium prepared from the host cells or organisms containing an expression vector which contains a nucleic acid insert encoding the desired protein or polypeptide is compared to medium prepared from the control cells or organism. The presence of a band in medium from the cells containing the nucleic

unlabeled proteins or polypeptides may be incubated with the cells and detected with antibodies having a detectable label, such as a fluorescent molecule, attached thereto.

Specificity of cell surface binding may be analyzed by conducting a competition analysis in which various amounts of unlabeled protein or polypeptide are incubated along with the labeled protein or polypeptide. The amount of labeled protein or polypeptide bound to the cell surface decreases as the amount of competitive unlabeled protein or polypeptide increases. As a control, various amounts of an unlabeled protein or polypeptide unrelated to the labeled protein or polypeptide is included in some binding reactions. The amount of labeled protein or polypeptide bound to the cell surface does not decrease in binding reactions containing increasing amounts of unrelated unlabeled protein, indicating that the protein or polypeptide encoded by the nucleic acid binds specifically to the cell surface.

As discussed above, human proteins have been shown to have a number of important physiological effects and, consequently, represent a valuable therapeutic resource. The human proteins or polypeptides made as described above may be evaluated to determine their physiological activities as described below.

EXAMPLE 24

Assaying the Expressed Proteins or Polypeptides for Cytokine,
Cell Proliferation or Cell Differentiation Activity

As discussed above, some human proteins act as cytokines or may affect cellular proliferation or differentiation. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein or polypeptide of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M$^-$ (preB M$^-$), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7c and CMK. The proteins or polypeptides prepared as described above may be evaluated for their ability to regulate T cell or thymocyte proliferation in assays such as those described above or in the following references: *Current Protocols in Immunology*, Ed. by J.E. Coligan *et al.*, Greene Publishing Associates and Wiley-Interscience; Takai *et al. J. Immunol.* **137**:3494-3500, 1986., Bertagnolli *et al.* J. Immunol. **145**:1706-1712, 1990., Bertagnolli *et al.*, *Cellular Immunology* **133**:327-341, 1991. Bertagnolli, *et al. J. Immunol.* **149**:3778-3783, 1992; and Bowman *et al., J. Immunol.* **152**:1756-1761, 1994, the entire disclosures of which are incorporated herein by reference.

activity to influence thymocyte or splenocyte cytotoxicity. Numerous assays for such activity are familiar to those skilled in the art including the assays described in the following references: Chapter 3 (*In vitro* Assays for Mouse Lymphocyte Function 3.1-3.19) and Chapter 7 (Immunologic studies in Humans) in *Current Protocols in Immunology* , J.E. Coligan *et al.* Eds, Greene Publishing Associates and Wiley-Interscience; Herrmann *et al., Proc. Natl. Acad. Sci. USA* **78**:2488-2492, 1981; Herrmann *et al., J. Immunol.* **128**:1968-1974, 1982; Handa *et al., J. Immunol.* **135**:1564-1572, 1985; Takai *et al., J. Immunol.* **137**:3494-3500, 1986; Takai *et al., J. Immunol.* **140**:508-512, 1988; Bowman *et al., J. Virology* **61**:1992-1998; Bertagnolli *et al. Cell. Immunol.* **133**:327-341, 1991; and Brown *et al., J. Immunol.* **153**:3079-3092, 1994, the entire disclosures of which are incorporated herein by reference.

The proteins or polypeptides prepared as described above may also be evaluated for their effects on T-cell dependent immunoglobulin responses and isotype switching. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references: Maliszewski, *J. Immunol.* **144**:3028-3033, 1990, the entire disclosure of which is incorporated herein by reference; and Mond *et al.* in *Current Protocols in Immunology*, **1** : 3.8.1-3.8.16, *supra*.

The proteins or polypeptides prepared as described above may also be evaluated for their effect on immune effector cells, including their effect on Th1 cells and cytotoxic lymphocytes. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references: Chapter 3 (*In vitro* Assays for Mouse Lymphocyte Function 3.1-3.19) and Chapter 7 (Immunologic Studies in Humans) in *Current Protocols in Immunology, supra*; Takai *et al., J. Immunol.* **137**:3494-3500, 1986; Takai *et al., J. Immunol.* **140**:508-512, 1988; and Bertagnolli *et al., J. Immunol.* **149**:3778-3783, 1992, the entire disclosures of which are incorporated herein by reference.

The proteins or polypeptides prepared as described above may also be evaluated for their effect on dendritic cell mediated activation of naive T-cells. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references: Guery *et al., J. Immunol.* **134**:536-544, 1995; Inaba *et al., J. Exp. Med.* **173**:549-559, 1991; Macatonia *et al., J. Immunol.* **154**:5071-5079, 1995; Porgador *et al J. Exp. Med* **182**:255-260, 1995; Nair *et al., J. Virol.* **67**:4062-4069, 1993; Huang *et al., Science* **264**:961-965, 1994; Macatonia *et al J. Exp. Med* **169**:1255-1264, 1989; Bhardwaj *et al., Journal of Clinical Investigation* **94**:797-807, 1994; and Inaba *et al., J. Exp. Med* **172**:631-640, 1990, the entire disclosures of which are incorporated herein by reference.

The proteins or polypeptides prepared as described above may also be evaluated for their influence on the lifetime of lymphocytes. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references: Darzynkiewicz *et al.*, *Cytometry* **13**:795-808, 1992; Gorczyca *et al.*, *Leukemia* 7:659-670, 1993; Gorczyca *et al.*, *Cancer Res.* **53**:1945-1951, 1993; Itoh *et al.*, *Cell* **66**:233-243, 1991; Zacharchuk, *J. Immunol.* **145**:4037-4045, 1990; Zamai *et al.*, *Cytometry* **14**:891-897, 1993; and Gorczyca *et al.*, *Int. J. Oncol.* 1:639-648, 1992, the entire disclosures of which are incorporated herein by reference.

The proteins or polypeptides prepared as described above may also be evaluated for their influence on early steps of T-cell commitment and development. Numerous assays for such activity are familiar to those skilled in the art, including without limitation the assays disclosed in the following references: Antica *et al.*, *Blood* **84**:111-117, 1994; Fine *et al.*, *Cell. Immunol.* **155**:111-122, 1994; Galy *et al.*, *Blood* **85**:2770-2778, 1995; and Toki *et al.*, *Proc. Nat. Acad Sci. USA* **88**:7548-7551, 1991, the entire disclosures of which are incorporated herein by reference.

Those proteins or polypeptides which exhibit activity as immune system regulators activity may then be formulated as pharmaceuticals and used to treat clinical conditions in which regulation of immune activity is beneficial. For example, the protein or polypeptide may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using the protein or polypeptide including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., plamodium. and various fungal infections such as candidiasis. Of course, in this regard, a protein or polypeptide may also be useful where a boost to the immune system generally may be desirable, *i.e.*, in the treatment of cancer.

Alternatively, the proteins or polypeptides prepared as described above may be used in treatment of autoimmune disorders including, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein or polypeptide may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which

immune suppression is desired (including, for example, organ transplantation), may also be treatable using the protein or polypeptide.

Using the proteins or polypeptides of the invention it may also be possible to regulate immune responses either up or down. Down regulation may involve inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T-cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active non-antigen-specific process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after the end of exposure to the tolerizing agent. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions, such as, for example, B7 costimulation), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking antibody), prior to transplantation, can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive

the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein or polypeptide of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. *De novo* bone synthesis induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein or polypeptide of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the proteins or polypeptides encoded by the nucleic acids described above is tendon/ligament formation. A protein or polypeptide encoded by the nucleic acids described above, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a protein or polypeptide of the present invention contributes to the repair of tendon or ligaments defects of congenital, traumatic or other origin and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The proteins or polypeptides of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors ex vivo for return *in vivo* to effect tissue repair. The proteins or polypeptides of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The therapeutic compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The proteins or polypeptides of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.*, for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders,

EXAMPLE 30

Assaying the Expressed Proteins or Polypeptides for Regulation of Blood Clotting

The proteins or polypeptides of the present invention may also be evaluated for their effects on blood clotting. Numerous assays for such activity are familiar to those skilled in the art, including the assays disclosed in the following references: Linet *et al., J. Clin. Pharmacol.* **26**:131-140, 1986; Burdick *et al., Thrombosis Res.* **45**:413-419, 1987; Humphrey *et al., Fibrinolysis* **5**:71-79 (1991); and Schaub, *Prostaglandins* **35**:467-474, 1988, the entire disclosures of which are incorporated herein by reference.

Those proteins or polypeptides which are involved in the regulation of blood clotting may then be formulated as pharmaceuticals and used to treat clinical conditions in which regulation of blood clotting is beneficial. For example, a protein or polypeptide of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein or polypeptide is expected to be useful in treatment of various coagulations disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein or polypeptide of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as infarction of cardiac and central nervous system vessels (e.g., stroke)). Alternatively, as described in more detail below, nucleic acids encoding blood clotting activity proteins or polypeptides or nucleic acids regulating the expression of such proteins or polypeptides may be introduced into appropriate host cells to increase or decrease the expression of the proteins or polypeptides as desired.

EXAMPLE 31

Assaying the Expressed Proteins or Polypeptides for Involvement in Receptor/Ligand Interactions

The proteins or polypeptides of the present invention may also be evaluated for their involvement in receptor/ligand interactions. Numerous assays for such involvement are familiar to those skilled in the art, including the assays disclosed in the following references: Chapter 7. 7.28.1-7.28.22) in *Current Protocols in Immunology*, J.E. Coligan *et al.* Eds. Greene Publishing Associates and Wiley-Interscience; Takai *et al., Proc. Natl. Acad. Sci. USA* **84**:6864-6868, 1987; Bierer *et al., J. Exp. Med.* **168**:1145-1156, 1988; Rosenstein *et al., J. Exp. Med.* **169**:149-160, 1989; Stoltenborg *et al., J. Immunol. Methods* **175**:59-68, 1994; Stitt *et al., Cell* **80**:661-670, 1995; and Gyuris *et al., Cell* **75**:791-803, 1993, the entire disclosures of which are incorporated herein by reference.

EXAMPLE 33

Assaying the Expressed Proteins or Polypeptides for Tumor Inhibition Activity

The proteins or polypeptides of the present invention may also be evaluated for tumor inhibition activity. In addition to the activities described above for immunological treatment or prevention of tumors, a protein or polypeptide of the invention may exhibit other anti-tumor activities. A protein or polypeptide may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein or polypeptide may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth. . Alternatively, as described in more detail below, nucleic acids encoding proteins or polypeptides with tumor inhibition activity or nucleic acids regulating the expression of such proteins or polypeptides may be introduced into appropriate host cells to increase or decrease the expression of the proteins or polypeptides as desired.

A protein or polypeptide of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein. Alternatively, as described in more detail below, nucleic acids encoding proteins or polypeptides

Proteins, polypeptides or other molecules interacting with proteins or polypeptides of the present invention can be found by a variety of additional techniques. In one method, affinity columns containing the protein or polypeptide of the present invention can be constructed. In some versions, of this method the affinity column contains chimeric proteins in which the protein or polypeptide of the present invention is fused to glutathione S-transferase. A mixture of cellular proteins or pool of expressed proteins as described above and is applied to the affinity column. Molecules interacting with the protein or polypeptide attached to the column can then be isolated and analyzed on 2-D electrophoresis gel as described in Ramunsen *et al. Electrophoresis*, **18**, 588-598 (1997). Alternatively, the molecules retained on the affinity column can be purified by electrophoresis based methods and sequenced. The same method can be used to isolate antibodies, to screen phage display products, or to screen phage display human antibodies.

Molecules interacting with the proteins or polypeptides of the present invention can also be screened by using an Optical Biosensor as described in Edwards & Leatherbarrow, *Analytical Biochemistry*, **246**, 1-6 (1997), the entire disclosure of which is incorporated herein by reference. The main advantage of the method is that it allows the determination of the association rate between the protein or polypeptide and other interacting molecules. Thus, it is possible to specifically select interacting molecules with a high or low association rate. Typically a target molecule is linked to the sensor surface (through a carboxymethl dextran matrix) and a sample of test molecules is placed in contact with the target molecules. The binding of a test molecule to the target molecule causes a change in the refractive index and/ or thickness. This change is detected by the Biosensor provided it occurs in the evanescent field (which extends a few hundred nanometers from the sensor surface). In these screening assays, the target molecule can be one of the proteins or polypeptides of the present invention and the test sample can be a collection of proteins, polypeptides or other molecules extracted from tissues or cells, a pool of expressed proteins, combinatorial peptide and/ or chemical libraries, or phage displayed peptides. The tissues or cells from which the test molecules are extracted can originate from any species.

In other methods, a target protein or polypeptide is immobilized and the test population is a collection of unique proteins or polypeptides of the present invention.

To study the interaction of the proteins or polypeptides of the present invention with drugs, the microdialysis coupled to HPLC method described by Wang *et al.*, *Chromatographia*, **44**, 205-208(1997) or the affinity capillary electrophoresis method described by Busch *et al.*, *J. Chromatogr.* 777:311-328 (1997), the entire disclosures of which are incorporated herein by reference, can be used.

nucleic acid in the sample is denatured and the PCR primers are specifically hybridized to complementary nucleic acid sequences in the sample. The hybridized primers are extended. Thereafter, another cycle of denaturation, hybridization, and extension is initiated. The cycles are repeated multiple times to produce an amplified fragment containing the nucleic acid sequence between the primer sites.

EXAMPLE 37

Use of the EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids as probes

Probes derived from EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids may be labeled with detectable labels familiar to those skilled in the art, including radioisotopes and non-radioactive labels, to provide a detectable probe. The detectable probe may be single stranded or double stranded and may be made using techniques known in the art, including *in vitro* transcription, nick translation, or kinase reactions. A nucleic acid sample containing a sequence capable of hybridizing to the labeled probe is contacted with the labeled probe. If the nucleic acid in the sample is double stranded, it may be denatured prior to contacting the probe. In some applications, the nucleic acid sample may be immobilized on a surface such as a nitrocellulose or nylon membrane. The nucleic acid sample may comprise nucleic acids obtained from a variety of sources, including genomic DNA, cDNA libraries, RNA, or tissue samples.

Procedures used to detect the presence of nucleic acids capable of hybridizing to the detectable probe include well known techniques such as Southern blotting, Northern blotting, dot blotting, colony hybridization, and plaque hybridization. In some applications, the nucleic acid capable of hybridizing to the labeled probe may be cloned into vectors such as expression vectors, sequencing vectors, or *in vitro* transcription vectors to facilitate the characterization and expression of the hybridizing nucleic acids in the sample. For example, such techniques may be used to isolate and clone sequences in a genomic library or cDNA library which are capable of hybridizing to the detectable probe as described in Example 20 above.

PCR primers made as described in Example 36 above may be used in forensic analyses, such as the DNA fingerprinting techniques described in Examples 38-42 below. Such analyses may utilize detectable probes or primers based on the sequences of the EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids.

contains 200 amplified sequences. This PCR-generated DNA is then digested with one or a combination of, preferably, four base specific restriction enzymes. Such enzymes are commercially available and known to those of skill in the art. After digestion, the resultant gene fragments are size separated in multiple duplicate wells on an agarose gel and transferred to nitrocellulose using Southern blotting techniques well known to those with skill in the art. For a review of Southern blotting see Davis *et al.* (Basic Methods in Molecular Biology, 1986, Elsevier Press. pp 62-65), the entire disclosure of which is incorporated herein by reference.

A panel of probes based on the sequences of the EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids are radioactively or colorimetrically labeled using methods known in the art, such as nick translation or end labeling, and hybridized to the Southern blot using techniques known in the art (Davis *et al.*, supra). Preferably, the probe is at least 10, 12, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400 or 500 nucleotides in length. Preferably, the probes are at least 10, 12, 15, 18, 20, 25, 28, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400 or 500 nucleotides in length. In some embodiments, the probes are oligonucleotides which are 40 nucleotides in length or less.

Preferably, at least 5 to 10 of these labeled probes are used, and more preferably at least about 20 or 30 are used to provide a unique pattern. The resultant bands appearing from the hybridization of a large sample of EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids will be a unique identifier. Since the restriction enzyme cleavage will be different for every individual, the band pattern on the Southern blot will also be unique. Increasing the number of probes will provide a statistically higher level of confidence in the identification since there will be an increased number of sets of bands used for identification.

EXAMPLE 41

Dot Blot Identification Procedure

Another technique for identifying individuals using the EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids disclosed herein utilizes a dot blot hybridization technique.

Genomic DNA is isolated from nuclei of subject to be identified. Probes are prepared that correspond to at least 10, preferably 50 sequences from the EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids. The probes are used to hybridize to the genomic DNA through conditions known to those in the art. The oligonucleotides are end labeled with P^{32} using polynucleotide kinase (Pharmacia). Dot

Blots are created by spotting the genomic DNA onto nitrocellulose or the like using a vacuum dot blot manifold (BioRad, Richmond California). The nitrocellulose filter containing the genomic sequences is baked or UV linked to the filter, prehybridized and hybridized with labeled probe using techniques known in the art (Davis *et al.*, *supra*). The ^{32}P labeled DNA fragments are sequentially hybridized with successively stringent conditions to detect minimal differences between the 30 bp sequence and the DNA. Tetramethylammonium chloride is useful for identifying clones containing small numbers of nucleotide mismatches (Wood *et al.*, *Proc. Natl. Acad. Sci. USA* **82(6)**:1585-1588 (1985), the entire disclosure of which is incorporated herein by reference). A unique pattern of dots distinguishes one individual from another individual.

EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids can be used as probes in the following alternative fingerprinting technique. In some embodiments, the probes are oligonucleotides which are 40 nucleotides in length or less.

Preferably, a plurality of probes having sequences from different EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids are used in the alternative fingerprinting technique. Example 42 below provides a representative alternative fingerprinting procedure in which the probes are derived from EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids.

EXAMPLE 42

Alternative "Fingerprint" Identification Technique

Oligonucleotides are prepared from a large number, e.g. 50, 100, or 200, EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids using commercially available oligonucleotide services such as Genset, Paris, France. Preferably, the oligonucleotides are at least 10, 15, 18, 20, 23, 25 28, or 30 nucleotides in length. However, in some embodiments, the oligonucleotides may be more than 40, 50, 60 or 70 nucleotides in length.

Cell samples from the test subject are processed for DNA using techniques well known to those with skill in the art. The nucleic acid is digested with restriction enzymes such as EcoRI and XbaI. Following digestion, samples are applied to wells for electrophoresis. The procedure, as known in the art, may be modified to accommodate polyacrylamide electrophoresis, however in this example, samples containing 5 ug of DNA are loaded into wells and separated on 0.8% agarose gels. The gels are transferred onto nitrocellulose using standard Southern blotting techniques.

10 ng of each of the oligonucleotides are pooled and end-labeled with P^{32}. The nitrocellulose is prehybridized with blocking solution and hybridized with the labeled probes. Following hybridization and washing, the nitrocellulose filter is exposed to X-Omat AR X-ray film. The resulting hybridization pattern will be unique for each individual.

It is additionally contemplated within this example that the number of probe sequences used can be varied for additional accuracy or clarity.

In addition to their applications in forensics and identification, EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids may be mapped to their chromosomal locations. Example 41 below describes radiation hybrid (RH) mapping of human chromosomal regions using EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids. Example 42 below describes a representative procedure for mapping EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids to their locations on human chromosomes. Example 43 below describes mapping of EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids on metaphase chromosomes by Fluorescence In Situ Hybridization (FISH).

2. Use of EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids in Chromosome Mapping

EXAMPLE 43

Radiation hybrid mapping of EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids to the human genome

Radiation hybrid (RH) mapping is a somatic cell genetic approach that can be used for high resolution mapping of the human genome. In this approach, cell lines containing one or more human chromosomes are lethally irradiated, breaking each chromosome into fragments whose size depends on the radiation dose. These fragments are rescued by fusion with cultured rodent cells, yielding subclones containing different portions of the human genome. This technique is described by Benham *et al.* (*Genomics* 4:509-517, 1989) and Cox *et al.*, (*Science* 250:245-250, 1990) , the entire disclosures of which are incorporated herein by reference. The random and independent nature of the subclones permits efficient mapping of any human genome marker. Human DNA isolated from a panel of 80-100 cell lines provides a mapping reagent for ordering EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related

nucleic acids. In this approach, the frequency of breakage between markers is used to measure distance, allowing construction of fine resolution maps as has been done using conventional ESTs (Schuler *et al.*, *Science* **274**:540-546, 1996), the entire disclosure of which is incorporated herein by reference.

RH mapping has been used to generate a high-resolution whole genome radiation hybrid map of human chromosome 17q22-q25.3 across the genes for growth hormone (GH) and thymidine kinase (TK) (Foster *et al.*, *Genomics* **33**:185-192, 1996), the region surrounding the Gorlin syndrome gene (Obermayr *et al.*, *Eur. J. Hum. Genet.* **4**:242-245, 1996), 60 loci covering the entire short arm of chromosome 12 (Raeymaekers *et al.*, *Genomics* **29**:170-178, 1995), the region of human chromosome 22 containing the neurofibromatosis type 2 locus (Frazer *et al.*, *Genomics* **14**:574-584, 1992) and 13 loci on the long arm of chromosome 5 (Warrington *et al.*, *Genomics* **11**:701-708, 1991), the entire disclosures of which are incorporated herein by reference.

EXAMPLE 44

Mapping of EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids to Human Chromosomes using PCR techniques

EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids may be assigned to human chromosomes using PCR based methodologies. In such approaches, oligonucleotide primer pairs are designed from EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids to minimize the chance of amplifying through an intron. Preferably, the oligonucleotide primers are 18-23 bp in length and are designed for PCR amplification. The creation of PCR primers from known sequences is well known to those with skill in the art. For a review of PCR technology see Erlich. in PCR Technology; Principles and Applications for DNA Amplification. 1992. W.H. Freeman and Co., New York, the entire disclosure of which is incorporated herein by reference.

The primers are used in polymerase chain reactions (PCR) to amplify templates from total human genomic DNA. PCR conditions are as follows: 60 ng of genomic DNA is used as a template for PCR with 80 ng of each oligonucleotide primer, 0.6 unit of Taq polymerase, and 1 μCu of a 32P-labeled deoxycytidine triphosphate. The PCR is performed in a microplate thermocycler (Techne) under the following conditions: 30 cycles of 94°C, 1.4 min; 55°C, 2 min; and 72°C, 2 min; with a final extension at 72°C for 10 min. The amplified products are analyzed on a 6% polyacrylamide sequencing gel and visualized by autoradiography. If the length of the resulting PCR product is

In a preferred embodiment, chromosomal localization of EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids are obtained by FISH as described by Cherif *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, 87:6639-6643, 1990, the entire disclosure of which is incorporated herein by reference). Metaphase chromosomes are prepared from phytohemagglutinin (PHA)-stimulated blood cell donors. PHA-stimulated lymphocytes from healthy males are cultured for 72 h in RPMI-1640 medium. For synchronization, methotrexate (10 μM) is added for 17 h, followed by addition of 5-bromodeoxyuridine (5-BrdU, 0.1 mM) for 6 h. Colcemid (1 μg/ml) is added for the last 15 min before harvesting the cells. Cells are collected, washed in RPMI, incubated with a hypotonic solution of KCl (75 mM) at 37°C for 15 min and fixed in three changes of methanol:acetic acid (3:1). The cell suspension is dropped onto a glass slide and air dried. The EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids is labeled with biotin-16 dUTP by nick translation according to the manufacturer's instructions (Bethesda Research Laboratories, Bethesda, MD), purified using a Sephadex G-50 column (Pharmacia, Upsala, Sweden) and precipitated. Just prior to hybridization, the DNA pellet is dissolved in hybridization buffer (50% formamide, 2 X SSC, 10% dextran sulfate, 1 mg/ml sonicated salmon sperm DNA, pH 7) and the probe is denatured at 70°C for 5-10 min.

Slides kept at -20°C are treated for 1 h at 37°C with RNase A (100 μg/ml), rinsed three times in 2 X SSC and dehydrated in an ethanol series. Chromosome preparations are denatured in 70% formamide, 2 X SSC for 2 min at 70°C, then dehydrated at 4°C. The slides are treated with proteinase K (10 μg/100 ml in 20 mM Tris-HCl, 2 mM $CaCl_2$) at 37°C for 8 min and dehydrated. The hybridization mixture containing the probe is placed on the slide, covered with a coverslip, sealed with rubber cement and incubated overnight in a humid chamber at 37°C. After hybridization and post-hybridization washes, the biotinylated probe is detected by avidin-FITC and amplified with additional layers of biotinylated goat anti-avidin and avidin-FITC. For chromosomal localization, fluorescent R-bands are obtained as previously described (Cherif *et al.*, *supra.*). The slides are observed under a LEICA fluorescence microscope (DMRXA). Chromosomes are counterstained with propidium iodide and the fluorescent signal of the probe appears as two symmetrical yellow-green spots on both chromatids of the fluorescent R-band chromosome (red). Thus, a particular EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids may be localized to a particular cytogenetic R-band on a given chromosome.

nucleic acids or fragments of positional segments of EST-related nucleic acids or a primer corresponding to a sequence included in the cloning vector. The resulting double stranded DNA is transformed into bacteria. cDNAs containing the EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids are identified by colony PCR or colony hybridization.

Once the upstream genomic sequences have been cloned and sequenced as described above, prospective promoters and transcription start sites within the upstream sequences may be identified by comparing the sequences upstream of the EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids with databases containing known transcription start sites, transcription factor binding sites, or promoter sequences.

In addition, promoters in the upstream sequences may be identified using promoter reporter vectors as described in Example 54.

EXAMPLE 54

Identification of Promoters in Cloned Upstream Sequences

The genomic sequences upstream of the EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids are cloned into a suitable promoter reporter vector, such as the pSEAP-Basic, pSEAP-Enhancer, pβ-gal-Basic, pβ-gal-Enhancer, or pEGFP-1 Promoter Reporter vectors available from Clontech. Briefly, each of these promoter reporter vectors include multiple cloning sites positioned upstream of a reporter gene encoding a readily assayable protein such as secreted alkaline phosphatase, β-galactosidase, or green fluorescent protein. The sequences upstream of the EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids are inserted into the cloning sites upstream of the reporter gene in both orientations and introduced into an appropriate host cell. The level of reporter protein is assayed and compared to the level obtained from a vector which lacks an insert in the cloning site. The presence of an elevated expression level in the vector containing the insert with respect to the control vector indicates the presence of a promoter in the insert. If necessary, the upstream sequences can be cloned into vectors which contain an enhancer for augmenting transcription levels from weak promoter sequences. A significant level of expression above that observed with the vector lacking an insert indicates that a promoter sequence is present in the inserted upstream sequence.

Appropriate host cells for the promoter reporter vectors may be chosen based on the results of the above described determination of expression patterns of the EST-related nucleic acids,

positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids. For example, if the expression pattern analysis indicates that the mRNA corresponding to a particular EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids is expressed in fibroblasts, the promoter reporter vector may be introduced into a human fibroblast cell line.

Promoter sequences within the upstream genomic DNA may be further defined by constructing nested deletions in the upstream DNA using conventional techniques such as Exonuclease III digestion. The resulting deletion fragments can be inserted into the promoter reporter vector to determine whether the deletion has reduced or obliterated promoter activity. In this way, the boundaries of the promoters may be defined. If desired, potential individual regulatory sites within the promoter may be identified using site directed mutagenesis or linker scanning to obliterate potential transcription factor binding sites within the promoter individually or in combination. The effects of these mutations on transcription levels may be determined by inserting the mutations into the cloning sites in the promoter reporter vectors.

EXAMPLE 55

Cloning and Identification of Promoters

Using the method described in Example 54 above with 5' ESTs, sequences upstream of several genes were obtained. Using the primer pairs GGG AAG ATG GAG ATA GTA TTG CCT G (SEQ ID NO:15) and CTG CCA TGT ACA TGA TAG AGA GAT TC (SEQ ID NO:16), the promoter having the internal designation P13H2 (SEQ ID NO:17) was obtained.

Using the primer pairs GTA CCA GGG ACT GTG ACC ATT GC (SEQ ID NO:18) and CTG TGA CCA TTG CTC CCA AGA GAG (SEQ ID NO:19), the promoter having the internal designation P15B4 (SEQ ID NO:20) was obtained.

Using the primer pairs CTG GGA TGG AAG GCA CGG TA (SEQ ID NO:21) and GAG ACC ACA CAG CTA GAC AA (SEQ ID NO:22), the promoter having the internal designation P29B6 (SEQ ID NO:23) was obtained.

Figure 4 provides a schematic description of the promoters isolated and the way they are assembled with the corresponding 5' tags. The upstream sequences were screened for the presence of motifs resembling transcription factor binding sites or known transcription start sites using the computer program MatInspector release 2.0, August 1996.

The transcription factor binding sites present in each of these promoters are listed as protein binding sites in the sequence listing for SEQ ID NOs:17, 20, and 23

Alternatively, oligonucleotides which are complementary to the strand normally transcribed in the cell may be synthesized *in vitro*. Thus, the antisense nucleic acids are complementary to the corresponding mRNA and are capable of hybridizing to the mRNA to create a duplex. In some embodiments, the antisense sequences may contain modified sugar phosphate backbones to increase stability and make them less sensitive to RNase activity. Examples of modifications suitable for use in antisense strategies are described by Rossi *et al.*, *Pharmacol. Ther.* **50(2)**:245-254, (1991), the entire disclosure of which is incorporated herein by reference.

Various types of antisense oligonucleotides complementary to the sequence of the EST-related nucleic acids, positional segments of EST-related nucleic acids or fragments of positional segments of EST-related nucleic acids may be used. In one preferred embodiment, stable and semi-stable antisense oligonucleotides described in International Application No. PCT WO94/23026, the entire disclosure of which is incorporated herein by reference, are used. In these molecules, the 3' end or both the 3' and 5' ends are engaged in intramolecular hydrogen bonding between complementary base pairs. These molecules are better able to withstand exonuclease attacks and exhibit increased stability compared to conventional antisense oligonucleotides.

In another preferred embodiment, the antisense oligodeoxynucleotides against herpes simplex virus types 1 and 2 described in International Application No. WO 95/04141, the entire disclosure of which is incorporated herein by reference, are used.

In yet another preferred embodiment, the covalently cross-linked antisense oligonucleotides described in International Application No. WO 96/31523, the entire disclosure of which is incorporated herein by reference, are used. These double- or single-stranded oligonucleotides comprise one or more, respectively, inter- or intra-oligonucleotide covalent cross-linkages, wherein the linkage consists of an amide bond between a primary amine group of one strand and a carboxyl group of the other strand or of the same strand, respectively, the primary amine group being directly substituted in the 2' position of the strand nucleotide monosaccharide ring, and the carboxyl group being carried by an aliphatic spacer group substituted on a nucleotide or nucleotide analog of the other strand or the same strand, respectively.

The antisense oligodeoxynucleotides and oligonucleotides disclosed in International Application No. WO 92/18522, the entire disclosure of which is incorporated herein by reference, may also be used. These molecules are stable to degradation and contain at least one transcription control recognition sequence which binds to control proteins and are effective as decoys therefor. These molecules may contain "hairpin" structures, "dumbbell" structures, "modified dumbbell" structures, "cross-linked" decoy structures and "loop" structures.

In some embodiments in which the protein or polypeptide is secreted, nucleic acids encoding the full length protein (*i.e.* the signal peptide and the mature protein), or nucleic acids encoding only the mature protein (*i.e.* the protein generated when the signal peptide is cleaved off) is introduced into the host organism.

The nucleic acids encoding the proteins or polypeptides may be introduced into the host organism using a variety of techniques known to those of skill in the art. For example, the extended cDNA may be injected into the host organism as naked DNA such that the encoded protein is expressed in the host organism, thereby producing a beneficial effect.

Alternatively, the nucleic acids encoding the protein or polypeptide may be cloned into an expression vector downstream of a promoter which is active in the host organism. The expression vector may be any of the expression vectors designed for use in gene therapy, including viral or retroviral vectors. The expression vector may be directly introduced into the host organism such that the encoded protein is expressed in the host organism to produce a beneficial effect. In another approach, the expression vector may be introduced into cells *in vitro*. Cells containing the expression vector are thereafter selected and introduced into the host organism, where they express the encoded protein or polypeptide to produce a beneficial effect.

EXAMPLE 60

Use of Signal Peptides To Import Proteins Into Cells

The short core hydrophobic region (h) of signal peptides encoded by the sequences of SEQ ID NOs. 24-728 and 766-792 may also be used as a carrier to import a peptide or a protein of interest, so-called cargo, into tissue culture cells (Lin *et al., J. Biol. Chem.*, **270**: 14225-14258 (1995); Du *et al., J. Peptide Res.*, **51**: 235-243 (1998); and Rojas *et al., Nature Biotech.*, **16**: 370-375 (1998), the entire disclosures of which are incorporated herein by reference).

When cell permeable peptides of limited size (approximately up to 25 amino acids) are to be translocated across cell membrane, chemical synthesis may be used in order to add the h region to either the C-terminus or the N-terminus to the cargo peptide of interest. Alternatively, when longer peptides or proteins are to be imported into cells, nucleic acids can be genetically engineered, using techniques familiar to those skilled in the art, in order to link the extended cDNA sequence encoding the h region to the 5' or the 3' end of a DNA sequence coding for a cargo polypeptide. Such genetically engineered nucleic acids are then translated either *in vitro* or *in vivo* after transfection into appropriate cells, using conventional techniques to produce the resulting cell permeable polypeptide. Suitable hosts cells are then simply incubated with the cell permeable polypeptide which is then translocated across the membrane.

This method may be applied to study diverse intracellular functions and cellular processes. For instance, it has been used to probe functionally relevant domains of intracellular proteins and to examine protein-protein interactions involved in signal transduction pathways (Lin *et al., supra*; Lin *et al., J. Biol. Chem.*, **271**: 5305-5308 (1996); Rojas *et al., J. Biol. Chem.*, **271**: 27456-27461 (1996); Liu *et al., Proc. Natl. Acad. Sci. USA*, **93**: 11819-11824 (1996); Rojas *et al., Bioch. Biophys. Res. Commun.*, **234**: 675-680 (1997), the entire disclosure of which is incorporated herein by reference).

Such techniques may be used in cellular therapy to import proteins producing therapeutic effects. For instance, cells isolated from a patient may be treated with imported therapeutic proteins and then re-introduced into the host organism.

Alternatively, the h region of signal peptides of the present invention could be used in combination with a nuclear localization signal to deliver nucleic acids into cell nucleus. Such oligonucleotides may be antisense oligonucleotides or oligonucleotides designed to form triple helixes, as described above, in order to inhibit processing and maturation of a target cellular RNA.

EXAMPLE 61

Computer Embodiments

As used herein the term "nucleic acid codes of SEQ ID NOs. 24-811 and 1600-1622 encompasses the nucleotide sequences of SEQ ID NOs. 24-811 and 1600-1622, fragments of SEQ ID NOs. 24-811 and 1600-1622, nucleotide sequences homologous to SEQ ID NOs. 24-811 and 1600-1622 or homologous to fragments of SEQ ID NOs. 24-811 and 1600-1622, and sequences complementary to all of the preceding sequences. The fragments include portions of SEQ ID NOs. 24-811 and 1600-1622 comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of SEQ ID NOs. 24-811 and 1600-1622. Preferably, the fragments are novel fragments. Preferably the fragments include polynucleotides described in Table II, polynucleotides described in Table III, polynucleotides described in Table IV or portions thereof comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of the polynucleotides described in Tables II, III, or IV. Homologous sequences and fragments of SEQ ID NOs. 24-811 and 1600-1622 refer to a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, or 75% homology to these sequences. Homology may be determined using any of the computer programs and parameters described in Example 18, including BLAST2N with the default parameters or with any modified parameters. Homologous sequences also include RNA sequences in which uridines replace the thymines in the nucleic acid codes of SEQ ID NOs. 24-811 and 1600-1622. The homologous sequences may be obtained using any of the procedures described herein or may result from the correction of a sequencing error as described above. Preferably the homologous sequences and fragments of SEQ ID NOs. 24-811 and 1600-1622 include polynucleotides described in Table II, polynucleotides described in Table III, polynucleotides described in Table IV or portions thereof comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of the polynucleotides described in Tables II, III, or IV. It will be appreciated that the nucleic acid codes of SEQ ID NOs. 24-811 and 1600-1622 can be represented in the traditional single character format (See the inside back cover of Stryer, Lubert. *Biochemistry*, 3rd edition. W. H Freeman & Co., New York.) or in any other format which records the identity of the nucleotides in a sequence.

As used herein the term "polypeptide codes of SEQ ID NOS. 812-1599" encompasses the polypeptide sequence of SEQ ID NOs. 812-1599 which are encoded by the 5' EST s of SEQ ID NOs. 24-811 and 1600-1622, polypeptide sequences homologous to the polypeptides of SEQ ID NOS. 812-1599, or fragments of any of the preceding sequences. Homologous polypeptide sequences refer to a polypeptide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, 75% homology to one of the polypeptide sequences of SEQ ID NOS. 812-1599. Homology

embodiment, the computer system is a Sun Enterprise 1000 server (Sun Microsystems, Palo Alto, CA). The computer system preferably includes a processor for processing, accessing and manipulating the sequence data. The processor can be any well-known type of central processing unit, such as the Pentium III from Intel Corporation, or similar processor from Sun, Motorola, Compaq or International Business Machines.

Preferably, the computer system is a general purpose system that comprises the processor and one or more internal data storage components for storing data, and one or more data retrieving devices for retrieving the data stored on the data storage components. A skilled artisan can readily appreciate that any one of the currently available computer systems are suitable.

In one particular embodiment, the computer system includes a processor connected to a bus which is connected to a main memory (preferably implemented as RAM) and one or more internal data storage devices, such as a hard drive and/or other computer readable media having data recorded thereon. In some embodiments, the computer system further includes one or more data retrieving device for reading the data stored on the internal data storage devices.

The data retrieving device may represent, for example, a floppy disk drive, a compact disk drive, a magnetic tape drive, etc. In some embodiments, the internal data storage device is a removable computer readable medium such as a floppy disk, a compact disk, a magnetic tape, etc. containing control logic and/or data recorded thereon. The computer system may advantageously include or be programmed by appropriate software for reading the control logic and/or the data from the data storage component once inserted in the data retrieving device.

The computer system includes a display which is used to display output to a computer user. It should also be noted that the computer system can be linked to other computer systems in a network or wide area network to provide centralized access to the computer system.

Software for accessing and processing the nucleotide sequences of the nucleic acid codes of SEQ ID NOs. 24-811 and 1600-1622, or the amino acid sequences of the polypeptide codes of SEQ ID NOS. 812-1599 (such as search tools, compare tools, and modeling tools etc.) may reside in main memory during execution.

In some embodiments, the computer system may further comprise a sequence comparer for comparing the above-described nucleic acid codes of SEQ ID NOs. 24-811 and 1600-1622 or polypeptide codes of SEQ ID NOS. 812-1599 stored on a computer readable medium to reference nucleotide or polypeptide sequences stored on a computer readable medium. A "sequence comparer" refers to one or more programs which are implemented on the computer system to compare a nucleotide or polypeptide sequence with other nucleotide or polypeptide sequences and/or compounds including but not limited to peptides, peptidomimetics, and chemicals stored within the

One embodiment is a process in a computer for determining whether two sequences are homologous. The process begins at a start state and then moves to a state wherein a first sequence to be compared is stored to a memory. The second sequence to be compared is then stored to a memory at a state. The process then moves to a state wherein the first character in the first sequence is read and then to a state wherein the first character of the second sequence is read. It should be understood that if the sequence is a nucleotide sequence, then the character would normally be either A, T, C, G or U. If the sequence is a protein sequence, then it should be in the single letter amino acid code so that the first and sequence sequences can be easily compared.

A determination is then made at a decision state whether the two characters are the same. If they are the same, then the process moves to a state wherein the next characters in the first and second sequences are read. A determination is then made whether the next characters are the same. If they are, then the process continues this loop until two characters are not the same. If a determination is made that the next two characters are not the same, the process moves to a decision state to determine whether there are any more characters either sequence to read.

If there are no more more characters to read, then the process moves to a state wherein the level of homology between the first and second sequences is displayed to the user. The level of homology is determined by calculating the proportion of characters between the sequences that were the same out of the total number of sequences in the first sequence. Thus, if every character in a first 100 nucleotide sequence aligned with a every character in a second sequence, the homology level would be 100%.

Alternatively, the computer program may be a computer program which compares the nucleotide sequences of the nucleic acid codes of the present invention, to reference nucleotide sequences in order to determine whether the nucleic acid code of SEQ ID NOs. 24-811 and 1600-1622 differs from a reference nucleic acid sequence at one or more positions. Optionally such a program records the length and identity of inserted, deleted or substituted nucleotides with respect to the sequence of either the reference polynucleotide or the nucleic acid code of SEQ ID NOs. 24-811 and 1600-1622. In one embodiment, the computer program may be a program which determines whether the nucleotide sequences of the nucleic acid codes of SEQ ID NOs. 24-811 and 1600-1622 contain a biallelic marker or single nucleotide polymorphism (SNP) with respect to a reference nucleotide sequence. This single nucleotide polymorphism may comprise a single base substitution, insertion, or deletion, while this biallelic marker may comprise about one to ten consecutive bases substituted, inserted or deleted.

The nucleic acid codes of SEQ ID NOs. 24-811 and 1600-1622 or the polypeptide codes of SEQ ID NOS. 812-1599 may be stored and manipulated in a variety of data processor programs in a variety of formats. For example, the nucleic acid codes of SEQ ID NOs. 24-811 and 1600-1622 or the polypeptide codes of SEQ ID NOS. 812-1599 may be stored as text in a word processing file, such as MicrosoftWORD or WORDPERFECT or as an ASCII file in a variety of database programs familiar to those of skill in the art, such as DB2, SYBASE, or ORACLE. In addition, many computer programs and databases may be used as sequence comparers, identifiers, or sources of reference nucleotide or polypeptide sequences to be compared to the nucleic acid codes of SEQ ID NOs. 24-811 and 1600-1622 or the polypeptide codes of SEQ ID NOS. 812-1599. The following list is intended not to limit the invention but to provide guidance to programs and databases which are useful with the nucleic acid codes of SEQ ID NOs. 24-811 and 1600-1622 or the polypeptide codes of SEQ ID NOS. 812-1599. The programs and databases which may be used include, but are not limited to: MacPattern (EMBL), DiscoveryBase (Molecular Applications Group), GeneMine (Molecular Applications Group), Look (Molecular Applications Group), MacLook (Molecular Applications Group), BLAST and BLAST2 (NCBI), BLASTN and BLASTX (Altschul *et al, J. Mol. Biol.* **215**: 403 (1990)), FASTA (Pearson and Lipman, *Proc. Natl. Acad. Sci. USA*, **85**: 2444 (1988)), FASTDB (Brutlag *et al.* Comp. App. Biosci. 6:237-245, 1990), Catalyst (Molecular Simulations Inc.), Catalyst/SHAPE (Molecular Simulations Inc.), Cerius^2.DBAccess (Molecular Simulations Inc.), HypoGen (Molecular Simulations Inc.), Insight II, (Molecular Simulations Inc.), Discover (Molecular Simulations Inc.), CHARMm (Molecular Simulations Inc.), Felix (Molecular Simulations Inc.), DelPhi, (Molecular Simulations Inc.), QuanteMM, (Molecular Simulations Inc.), Homology (Molecular Simulations Inc.), Modeler (Molecular Simulations Inc.), ISIS (Molecular Simulations Inc.), Quanta/Protein Design (Molecular Simulations Inc.), WebLab (Molecular Simulations Inc.), WebLab Diversity Explorer (Molecular Simulations Inc.), Gene Explorer (Molecular Simulations Inc.), SeqFold (Molecular Simulations Inc.), the EMBL/Swissprotein database, the MDL Available Chemicals Directory database, the MDL Drug Data Report data base, the Comprehensive Medicinal Chemistry database, Derwents's World Drug Index database, the BioByteMasterFile database, the Genbank database, and the Genseqn database. Many other programs and databases would be apparent to one of skill in the art given the present disclosure.

Motifs which may be detected using the above programs include sequences encoding leucine zippers, helix-turn-helix motifs, glycosylation sites, ubiquitination sites, alpha helices, and beta sheets, signal sequences encoding signal peptides which direct the secretion of the

encoded proteins, sequences implicated in transcription regulation such as homeoboxes, acidic stretches, enzymatic active sites, substrate binding sites, and enzymatic cleavage sites.

EXAMPLE 62

Methods of Making Nucleic Acids

The present invention also comprises methods of making the EST-related nucleic acids, fragments of EST-related nucleic acids, positional segments of the EST-related nucleic acids, or fragments of positional segments of the EST-related nucleic acids. The methods comprise sequentially linking together nucleotides to produce the nucleic acids having the preceding sequences. A variety of methods of synthesizing nucleic acids are known to those skilled in the art.

In many of these methods, synthesis is conducted on a solid support. These included the 3' phosphoramidite methods in which the 3' terminal base of the desired oligonucleotide is immobilized on an insoluble carrier. The nucleotide base to be added is blocked at the 5' hydroxyl and activated at the 3' hydroxyl so as to cause coupling with the immobilized nucleotide base. Deblocking of the new immobilized nucleotide compound and repetition of the cycle will produce the desired polynucleotide. Alternatively, polynucleotides may be prepared as described in U.S. Patent No. 5,049,656. In some embodiments, several polynucleotides prepared as described above are ligated together to generate longer polynucleotides having a desired sequence.

EXAMPLE 63

Methods of Making Polypeptides

The present invention also comprises methods of making the polynucleotides encoded by EST-related nucleic acids, fragments of EST-related nucleic acids, positional segments of the EST-related nucleic acids, or fragments of positional segments of the EST-related nucleic acids and methods of making the EST-related polypeptides, fragments of EST-related polypeptides, positional segments of EST-related polypeptides, or fragments of EST-related polypeptides. The methods comprise sequentially linking together amino acids to produce the nucleic polypeptides having the preceding sequences. In some embodiments, the polypeptides made by these methods are 150 amino acid or less in length. In other embodiments, the polypeptides made by these methods are 120 amino acids or less in length.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,822,072

DATED : November 23, 2004

INVENTORS : Jean-Baptiste Dumas Milne Edwards, Aymeric Duclert, Jean-Yves Giordano

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 2:
Line 28, "268:7314," should read --268:731-4,--.

Column 4:
Line 1, "6:236244," should read --6:236-244,--.
Line 3, "Spel" should read --SpeI--.
Line 4, "Spel" should read --SpeI--.

Column 5:
Line 19, "tenn" should read --term--.

Column 7:
Line 61, "extended,cDNAs" should read --extended cDNAs--.

Column 8:
Line 63, "SEQ D NOs." should read --SEQ ID NOs.--.

Column 12:
Line 35, "15541580" should read --1554-1580--.

Column 20:
Line 5, "gene D" should read --gene II--.
Line 21, "polyerase" should read --polymerase--.

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It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 21:
Line 38, "fill-length" should read --full-length--.

Column 25:
Line 8, "Ednian" should read --Edman--.
Line 9, "N-terrrinal" should read --N-terminal--.

Column 27:
Line 41, "24728" should read --24-728--.

Column 30:
Line 11, "nucleobde(s)" should read --nucleotide(s)--.

Column 31:
SEQ ID NO: 415, "53" should read --S3--.

Column 33:
SEQ ID NO: 800, "km" should read --krn--.

Column 34:
Line 50, "polynucleolide" should read --polynucleotide--.

Column 45:
Line 34, "5'ESTs Consensus" should read --5'ESTs, Consensus--.
Line 53, "the type" should read --the tissue type--.

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It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 54:
Lines 12-14, "647 F:1 should read --647 F:1
643 F:1 648 F:1
649 F:1" 649 F:1--.

Column 59:
Line 38, "by.,a" should read --by a--.

Column 60:
Line 41, "it'may" should read --it may--.

Column 61:
Line 53, "bioinfomnatics" should read --bioinformatics--.

Column 67:
Line 31, "403410" should read --403-410--.

Column 68:
Line 1, "17:4941" should read --17:49-61--.

Column 69:
Line 61, "10%-20%=80%" should read --100%-20%=80%--.

Column 70:
Line 63, "Gene H" should read --Gene II--.

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It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 72:
Line 29, "10:685686" should read --10:685-686--.

Column 75:
Line 6, "pX1 contains" should read --pXT1 contains--.
Line 16, "BgIII" should read --BG1II--.

Column 77:
Line 59, "Cytokine Cell" should read --Cytokine, Cell--.

Column 78:
Line 12, "145:17061712" should read --145:1706-1712--.

Column 79:
Line 36, "Takcai" should read --Takai--.
Line 44, "134:536544" should read --134:536-544--.
Line 47, "67:40624069" should read --67:4062-4069--.
Line 57, "Darzynliewicz" should read --Darzynkiewicz--.
Line 59, "7:659670" should read --7:659-670--.

Column 81:
Line 2, "tsplants" should read --transplants--.

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It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 85:
Line 11, "tendonaigament-like" should read --tendon/ligament-like--.
Line 20, "in viva" should read --in vivo--.

Column 87:
Line 55, "45:413419" should read --45:413-419--.
Line 57, "35:467474" should read --35:467-474--.

Column 89:
Line 50, "Inclining" should read --killing--.
Line 63, "behaviora" should read --behavioral--.

Column 91:
Line 48, "*Chromalographia*" should read --*Chromatographia*--.

Column 95:
Line 40, "GIC" should read --G/C--.
Line 62, "Acids Positional" should read --Acids, Positional--.

Column 97:
Line 49, "probes arc" should read --probes are--.

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INVENTORS : Jean-Baptiste Dumas Milne Edwards, Aymeric Duclert, Jean-Yves Giordano

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 98:
Line 11, "P2" should read --P^{32}--.
Line 18, "^{32}p" should read --^{32}P--.
Line 67, "p^{32}" should read --P^{32}--.

Column 99:
Line 64, "(FK)" should read --(TK)--.

Column 100:
Line 9, "Acids Positional" should read --Acids, Positional--.

Column 101:
Line 40, "Collected" should read --Colcemid--.

Column 112:
Line 23, "in, both" should read --in both--.

Column 113:
Line 11, "CIA" should read --CTA--.
Line 19, "release 20" should read --release 2.0--.

Column 115:
Line 66, "antiseese" should read --antisense--.

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It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 119:
Line 30, "region (b)" should read --region (h)--.
Line 61, "271:2745627461" should read --271:27456-27461--.
Line 63, "234:675680" should read --234:675-680--.

Column 120:
Line 56, "3^4 edition" should read --3^{rd} edition--.
Lines 66-67, "990%, 98%, 97%, 96% 95%, 900%, 85%" should read --99%, 98%, 97%, 96%, 95%, 90%, 85%--.

Column 122:
Line 18, "Continuing" should read --containing--.

Column 124:
Lines 60-61, "polynucleotides" should read --polynucleotide--.

Column 127:
Lines 39-40, "MicrosofRWORD" should read --MicrosoftWORD--.

Column 128:
Line 49, "desired polynucleotides." should read --desired polynucleotide.--.

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